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ACTION OF BETA-AMYLASE FROM SOYBEANS ON VARIOUS STARCHES [1]

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Previous work (Martin and Newton, 1938) has shown that when starches from different sources are heated at their optimum gelatinization temperatures and then cooled, beta-amylase action takes place at the same rate on all the starches. Since the series studied contained starches with both high and low phosphorus and fatty-acid content, the question of the importance of these groups in enzyme action arose. Furthermore, the appearance of a flocculent material in the digestion mixtures and the fact that the maltose formed accounted for only about 70% of the starch showed the presence of some interesting residual substances.

Mÿrback (1937) suggested that both the fatty acids and the phosphorus in starches might stop the enzyme action. Samec (1927) concluded that the phosphorus present in potato starch (0.015%) inhibits or blocks the action of the enzyme. On the other hand, Pringsheim and Ginsberg (1935) reported that complete hydrolysis of starch was obtained without liberating any free phosphoric acid. Taylor and Sherman (1933) concluded that lipase-free amylase could attack the linkages of fatty acids to the starch molecules. However, since Schoch (1938) was able to remove the fatty acids from corn starch by extraction methods, it is doubtful if a strictly chemical bond links the fatty acids to the starch.

The fact that a flocculent material appears in the enzyme digestion of starch was first noticed by Baker (1902). Other investigators (Fernbach and Wolff, 1904; Sallinger, 1919; Schryver and Thomas, 1923; Ling and Nanji, 1925; Hermano and Rask, 1926; Malloch, 1929; and Clayson and Schryver, 1923) have described the appearance of this material in amylase digestions. Sherman and Punnett (1916)

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filtered out the flocculent material and weighed it. They found that the amount (1.08–1.4%) was independent of the enzyme used.

Baker (1902), Syniewski (1925), Freeman and Hopkins (1936), Blom, Bak, and Braae (1936), and Hanes (1937) have shown that the increase in reducing action during the beta-amylase digestion of potato starch is due almost entirely to the formation of maltose. The limit of production of maltose is 60%–67% of the starch as confirmed by van Klinkenberg (1932), Samec (1935) and Hanes (1937). Work in this laboratory has shown that the same limit is reached in digestions of corn, wheat, rice, and tapioca starches as well.

Throughout the hydrolysis of starch by beta-amylase, residual starch-like substances are present which can be precipitated by 50%–60% alcohol. The material that is left at the end of the reaction, which should amount to 30%–35% of the starch on the basis of the maltose calculation, was separated by Wijsman (1890), who named it "erythrogranulose." Baker (1902) and Haworth, Hirst and Waine (1935) prepared it in about the same way and named it "alpha amylodextrin." A summary of the properties of this material as obtained by different investigators is given by Hanes (1937). The results are not at all in agreement.

That this material is a part of the original starch which the beta-amylase cannot attack seems more probable than that it is a product of a secondary reaction. Pringsheim and Beiser (1924) concluded that the 60% alcohol precipitate from beta-amylase digestions was an intact part of the original starch. Mÿrback (1937) considered that the enzyme could split maltose from all starch molecules but to varying degrees. The residual material would then consist of fragments of the original starch molecules which for some reason cannot be attacked by the amylase.

From the point of view of Mÿrback (1937) the variations in the reported properties of this material could be due to differences in the starches. On the basis of this concept the residual material from beta-amylase action on the different starches should contain that portion of the starch molecule which causes the difference in the various properties of the starches.

Preparation of Residual Materials

The general procedure followed in separating the residual materials from the beta-amylase digestions of different starches can be represented by Figure 1. Precipitates A and B were prepared from corn, wheat, rice, potato, and tapioca starches. The amylase was prepared from soybean meal by the method of Newton and Naylor (1939), who classified the enzyme as beta amylase from mutarotation studies.

The starches were gelatinized at the temperature found to be optimum for beta-amylase action (Martin and Newton, 1938). The substrates were prepared by the following method. Eight liters of a solution containing 980 cc. of 0.2M NaH_2PO_4 and 20 cc. of 0.2M Na_2HPO_4 was placed in a 16-liter balloon flask in a water bath and allowed to come to the temperature desired. Then 600 g. of untreated starch, suspended in 2 liters of water, was poured into the flask with stirring. After 30 minutes the flask was removed from the bath and cooled in running water, and placed in a thermostat at 40°C. One hundred cc. of a suspension containing 700 mg. of soybean amylase was added. The mixture was stirred vigorously and let stand 5

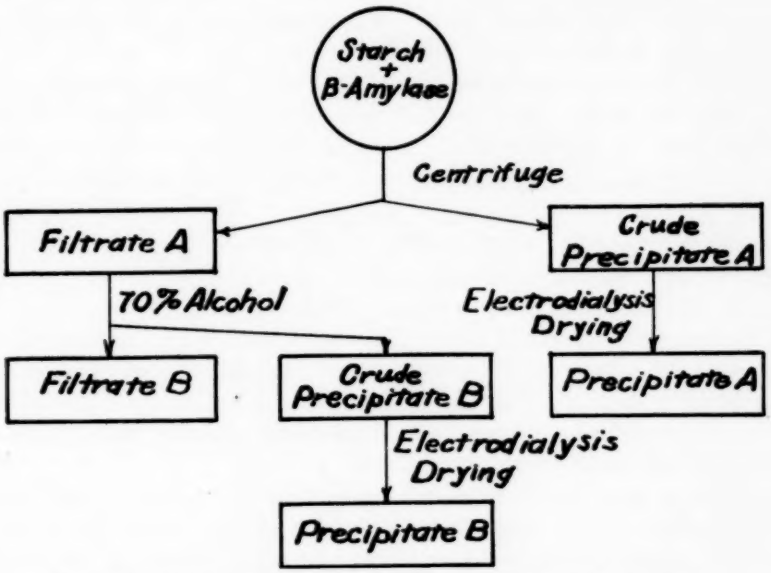

Fig. 1. Procedure followed in separating the residual materials from beta-amylase digestions of different starches.

hours. At the end of this time the flask was placed in a refrigerator for 16 hours. This procedure seemed to facilitate the removal of precipitate A. The digestion mixture was then run through a Sharples supercentrifuge. One-cc. samples were removed from the centrifugate for the sugar determination.

The solid material (precipitate A) which collected in the bowl of the centrifuge was shaken up in 1 liter of water. This suspension was electrodialyzed because the material could not be recovered from the wash water by supercentrifuging. When the material had settled in the dialyzer (after about 12 hours) the supernatant liquor was siphoned off. More water was added, the solids shaken up, and

dialysis continued. This process was repeated two or three times until the liquid from the anode chamber gave no test for phosphate.

Attempts to dry the precipitate in air at this point resulted in a dark-colored hard mass which could not be ground. Therefore, after dialysis the thick suspension from the bottom of the dialyzer was put into twice its volume of absolute alcohol and allowed to stand overnight. The supernatant liquid was siphoned off and another portion of absolute alcohol added. The solid material was again allowed to settle. This treatment was continued until the precipitate was sufficiently granular to filter with suction. Dehydration was completed by grinding under several portions of absolute alcohol. The material was then dried with ether and placed in a vacuum desiccator for two or three days. When dry, precipitate A was ground to a white powder in an agate mortar.

The fraction called precipitate B (Fig. 1) was prepared from five different starches. The precipitate was prepared by adding 2200 cc. of absolute alcohol to 1500 cc. of the centrifugate from the preparation of precipitate A. The mixture was allowed to settle and then centrifuged or the supernatant liquid siphoned off, depending on the nature of the precipitate. The appearance and nature of precipitate B were quite different from the cereal and root starches. When prepared from the cereal starches, it was curdy and settled out nicely, while if prepared from the root starches, it was formed as a transparent sticky mass. When the precipitate was flocculent, it was washed once with 60% alcohol, then dried by grinding under absolute alcohol. The gummy precipitates were repeatedly ground under absolute alcohol and dried to white powders.

These crude products were later redissolved in water and electrodialyzed until free from phosphate. The material was recovered as before. However, in the case of the material from potato and tapioca starches, electrodialysis was necessary to separate it from the alcohol mixtures. In this purification process, 50% to 75% of precipitate B was lost.

The yields of precipitates A and B from a series of starches are given in Table I. The yields of precipitate B are based on the amount of the crude product. The percentage of maltose formed is also given and is based directly on the total reducing value of the solution.

Characterization of Precipitates

The precipitates were characterized as to further hydrolysis by fresh beta-amylase, by phosphorus and fatty-acid content, by reducing power, and by iodine precipitation according to the procedure of Denny (1922). The results are recorded in Table II.

TABLE I

YIELDS OF SOME PRODUCTS OF BETA-AMYLASE ACTION

Kind of starch	Maltose equiv.	Precipitate A	Precipitate B	Total yields
	%	%	%	%
Corn	51.4	1.64	32.2	88.24
Rice	38.5	1.92	34.4	74.82
Wheat	51.8	1.0	38.8	91.6
Potato	55.1	0.84	30.0	85.94
Tapioca	55.6	0.05	33.5	89.15

TABLE II

PROPERTIES OF PRODUCTS OF BETA-AMYLASE ACTION

Substance	Percent yield	Enzymatic digestion, % maltose	Reducing power		Phosphorus		Fat		Starch[4]	
			Mg. maltose/g	R_{eu}[1]	%[2]	Recovery[3]	%[2]	Recovery[3]	Mg. I ads	% Stch
Corn starch	—	58.5	16.8	5.5	0.015	—	0.66	—	2.9	65.6
Ppt. A	1.64	14.1	27.3	9.6	0.031	3.38	1.31	3.26	3.25	69.0
Ppt. B	32.2	15.3	27.3	8.2	0.033	70.8	0.71	34.5	0.86	19.6
Rice starch	—	65.7	37.8	7.8	0.035	—	0.62	—	3.12	71.0
Ppt. A	1.92	30.0	48.3	11.2	0.041	2.25	0.95	2.90	3.9	89.2
Ppt. B	34.4	34.0	48.3	13.0	0.033	32.8	0.56	31.0	4.05	92.0
Wheat starch	—	60.0	25.2	10.4	0.051	—	0.57	—	3.15	71.6
Ppt. A	1.0	23.9	46.2	11.3	0.041	1.8	0.90	1.5	3.2	76.7
Ppt. B	38.8	26.2	46.2	13.0	0.152	103.0	0.91	51.4	4.0	90.5
Potato starch	—	59.3	16.8	4.3	0.050	—	0.076	—	3.5	79.5
Ppt. A	0.84	9.65	63.0	36.5	0.112	1.88	0.17	1.8	—	—
Ppt. B	30.0	9.02	63.0	13.8	0.222	102.5	0.18	71.0	—	—
Tapioca starch	—	77.2	33.6	4.3	0.010	—	0.174	—	2.95	67.0
Ppt. A	0.05	6.3	52.5	19.0	0.020	1.0	0.51	1.46	—	—
Ppt. B	33.5	9.8	33.6	14.6	0.020	67.0	0.22	42.3	—	—

[1] Reu determinations were made by B. Brimhall.
[2] Actual percentage present.
[3] The recovery of fat and phosphorus is expressed as percentage of the groups in the original starch and is based on the yields given in the first column.
[4] The adsorbed iodine method for determining starch according to Denny (1922).

For the further beta-amylase action, one-percent unbuffered substrates were used. The substrates were boiled. Five cc. of a suspension containing 40 mg. of soybean amylase in 50 cc. of water was added to 100 cc. of substrate. Five-cc. samples were removed at intervals for sugar determination. The original starches were tested in exactly the same way as the preparations.

Since the phosphorus content of these materials was very low, micro technique was used in analyzing for phosphorus. The volu-

metric method of Pregl (1937) was modified somewhat, in that the yellow precipitate was washed with 3% potassium-nitrate solution instead of alcohol. The solutions used were the same as in the Pregl method. The fatty-acid content of the original starches and the precipitates was determined by the method of Taylor and Nelson (1920). In the columns headed "recovery" in Table II under fat and phosphorus, the amount of these groups present in the preparations is expressed as percentage of these groups present in the original starches. The calculated recovery of fat and phosphorus is based on the yields given in the first column.

The reducing power of the preparations was measured both against ferricyanide (Martin and Newton, 1938) and against copper by the method of Farrow (Richardson, Higgenbotham, and Farrow, 1936). The determinations by the potentiometric method were made on 5.0 cc. of a one-percent suspension that had been boiled. The reducing value was calculated as mg. of maltose per gram of sample. The reducing power against copper is given as R_{cu} values according to Farrow.

The adsorbed-iodine method for the determination of starch (Denny, 1922) was used to compare the precipitates A and B with the original starches. The calculated percentage recovery is based on the factor (g. starch)/(g. iodine) = 0.11 (Denny, 1922) determined on soluble potato starch. The preparations from potato and tapioca starches gave the customary deep violet-black color, but no precipitate could be centrifuged or filtered out.

Discussion and Conclusions

The data summarized in Table II show that the precipitates are quite different from the original starches. The reducing power and phosphorus and fatty-acid contents are, in general, higher than in the original starches. This corresponds to the theory that these materials are fragments of the starch molecules and contain a concentration of the associated phosphorus and fatty-acid groups. The apparently anomalous results on the original starches with the starch determination of Denny are due to the use of the factor determined on soluble starch. The method is apparently not specific for unchanged starch.

If fractions A and B from each individual starch are compared, there appears to be no consistent difference in the chemical properties measured. The reducing power of precipitates A and B is very nearly the same, by either the potentiometric method or the copper method of Farrow. The fat content is slightly higher in precipitate A from corn, rice, and tapioca starches. The phosphorus content of precipitates A and B is about the same except for wheat and potato

starches, where precipitate B is much higher in phosphorus. In the starch determination the behavior of precipitates A and B from any one kind of starch is very similar. The only consistent difference between the two products from any one kind of starch is the fact that precipitate A flocculates directly from the digestion, while precipitate B comes out in 70% alcohol.

The differences in the original starches, particularly between the cereal and root starches, are magnified in the residual material from beta-amylase action. The reducing power of the residual materials from potato and tapioca starches is higher than of these precipitates from the cereal starches, but the residual materials from the root starches are not hydrolyzed as far by fresh beta-amylase. The fatty-acid contents of the precipitates from the digestions of root starches are lower than those from the cereal starches, but the difference between the residues from potato and from tapioca starches appears larger than that between the cereal and root starches. There are no significant trends in the phosphorus content. The starch determinations show a marked difference, which is probably due to the difference in physical nature of these materials as noted in their preparation. The differences in the residual materials from cereal and root starches are apparently of degree rather than kind and cannot be entirely explained on the basis of the phosphorus and fatty-acid content.

The role of the phosphorus and fatty acids is apparently unimportant in the beta-amylase digestion of starches. While the optimum temperatures for preparing the substrates may be correlated to the fatty-acid content, the importance of the phosphorus is much more obscure. When the different starches are prepared at their optimum temperatures, the rate of beta-amylase action is the same, and the digestion limits are about the same. Therefore, the blocking of the enzyme does not seem to be caused by the presence of these groups.

Summary

The non-digested portions of corn, wheat, rice, potato, and tapioca starches resulting from the action of soybean beta-amylase were used to prepare a flocculent, water-insoluble fraction and a gummy, water-soluble fraction precipitated by 70% alcohol.

These fractions have been characterized as to reducing power, further hydrolysis by beta-amylase, phosphorus and fatty-acid content, and precipitation by iodine according to the procedure of Denny.

The flocculent material and the alcohol precipitate from any one kind of starch are apparently very similar.

The physical natures of these preparations are very different, depending on whether they originate from cereal or root starches.

The phosphorus and fatty-acid groups do not appear to be the agents which block the action of beta amylase at 60%–70% conversion of starch to maltose.

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A NOTE ON MOISTURE INTERCHANGE IN MIXED WHEATS, WITH OBSERVATIONS ON THE RATE OF ABSORPTION OF MOISTURE BY WHEAT

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The observations recorded in this note were made in preliminary work in the large field of wheat conditioning and the moisture relationships of wheats. It is a common practice to partially dry excessively damp wheat by mixing it with a dry wheat and allowing the mixture to lie for a convenient length of time. It is well known that under such conditions the wheats tend to equalise in moisture content, and, if the wheats are suitably chosen, the resulting mixture may be in quite good condition for milling. Little, however, is known precisely as to the nature and extent of the process of moisture interchange involved.

The work here reported was done mainly on Karachi and English wheats—which are sometimes treated in this way by English millers. In the first experiment three quarters of an approximately 50–50 blend of English and mixed Karachi wheats, which had "natural" moisture

contents before mixing of 20.65% and 10.10% respectively, were allowed to remain in a three-quarter wooden zinc-lined bin for about six weeks. From time to time, daily at first, then at less frequent intervals, samples were taken, the grains of each of the two wheats were separated by hand picking, and moisture determinations made on each lot (results shown in Table I and Figure 1). It will be noticed

TABLE I

PRELIMINARY OBSERVATIONS ON MOISTURE INTERCHANGE IN WHEAT DURING STORAGE

Time in days from start	Moisture content of English wheat	Moisture content of Karachi wheat	Difference
	%	%	%
0	20.65 (before mixing)	10.10 (before mixing)	10.55
1	17.82	13.18	4.64
2	17.02	13.74	3.28
3	17.13	14.71	2.42
4	16.72	13.90	2.82
5	15.74	13.62	2.12
6	16.65	14.38	2.27
7	17.14	14.27	2.87
8	17.20	14.32	2.88
9	17.20	14.47	2.73
13	17.10	15.29	1.81
14	17.02	14.43	2.59
15	16.96	14.36	2.60
16	16.88	14.29	2.59
19	17.05	14.70	2.35
20	16.92	14.48	2.44
27	16.83	14.38	2.45
29	16.97	14.59	2.38
33	16.98	14.56	2.42
35	16.75	14.45	2.30
40	17.10	14.79	2.31
43	16.91	14.64	2.27
47	17.00	14.80	2.20
Mean of last 17 determinations	16.98	14.54	2.44

Mean moisture of unmixed wheats, 15.38%.
Mean of means of last 17 determinations, 15.76%.

that moisture interchange between the damp and dry wheats was very rapid during the first two days, while practically no change occurred after six days. From the third until the 47th day after mixing an approximately constant difference in moisture content of 2.4% persisted between the two wheats, and there appeared to be no tendency for this difference to decrease.

The results, striking as they are, are obviously subject to certain disturbing factors. In the first place, the mixing of the wheats had been done on the commercial scale by the power-driven mixers at the foot of the storage bins in a commercial mill, and there was no cer-

tainty therefore that the mixture was either accurately 50–50 or uniform. Again, under the conditions of storage, the mixture was likely to be affected by change in atmospheric conditions, although as shown by Table I, this effect was actually very small. The ready response of wheat to change in relative humidity is, however, well known.

Further tests were made under more precise conditions. A sample of red English wheat with a "natural" moisture content of 17.33% was sprayed evenly (to increase its moisture content) and allowed to stand several days. Immediately before the experiments its moisture content was 21.99%. In order to make the separation (by hand picking) of the mixtures to be tested easy and quick, white Karachi grains only were used. The white grains were separated by hand

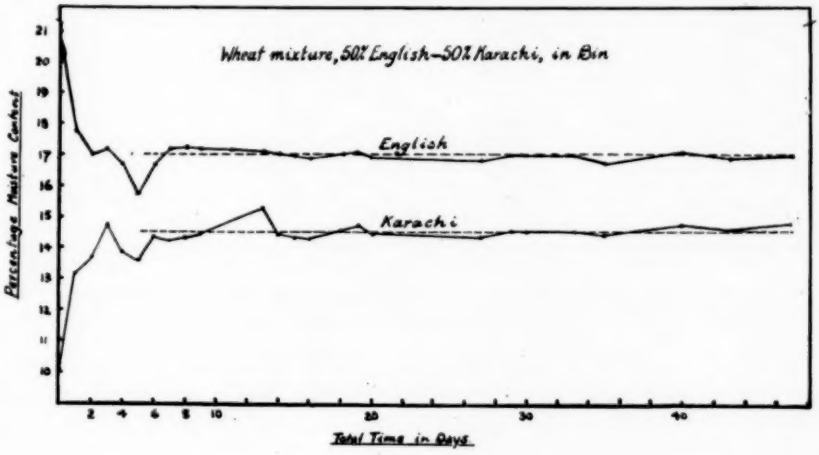

Figure 1

picking from a considerable sample of the original Karachi and were easily picked out after mixing with the red English grains. Immediately before use in the tests the moisture content of this well-mixed white Karachi was found to be 12.62%.

Several glass bulbs each capable of holding over 30 grams of wheat were blown and provided with necks through which wheat could be introduced; the necks were shaped to allow convenient sealing-off in the blow-pipe flame.

Fifteen grams of the English wheat was thoroughly mixed with 15 grams of the Karachi, and the mixture introduced into a glass bulb, which was then sealed in the blow-pipe flame.

Twelve bulbs were so prepared and immersed in a water bath, whose temperature was maintained at 20° C. At regular intervals of

time a bulb was removed, opened, the mixture separated, and moisture determinations made on the two components. The separation of the mixture by hand picking did not take longer than three or four minutes. The results are given in Table II and Figure 2.

TABLE II

MOISTURE INTERCHANGE IN A 50–50 MIXTURE OF DAMP ENGLISH AND DRY WHITE KARACHI WHEATS

Time in days from start	Moisture of English	Moisture of Karachi	Difference
	%	%	%
0 (before mixing)	21.99	12.62	9.37
1	18.49	15.54	2.95
2	18.09	16.32	1.77
3	18.05	16.53	1.42
4	17.86	16.34	1.52
5	18.02	16.63	1.39
7	18.02	16.91	1.11
9	17.87	16.82	1.05
11	18.00	16.99	1.01
17	17.81	16.59	1.22
21	17.98	16.93	1.05
25	17.75	16.73	1.02
Mean of last 7 determinations	17.92	16.80	1.12

Mean moisture of unmixed wheats, 17.31%.
Mean of means of last 7 determinations, 17.36%.

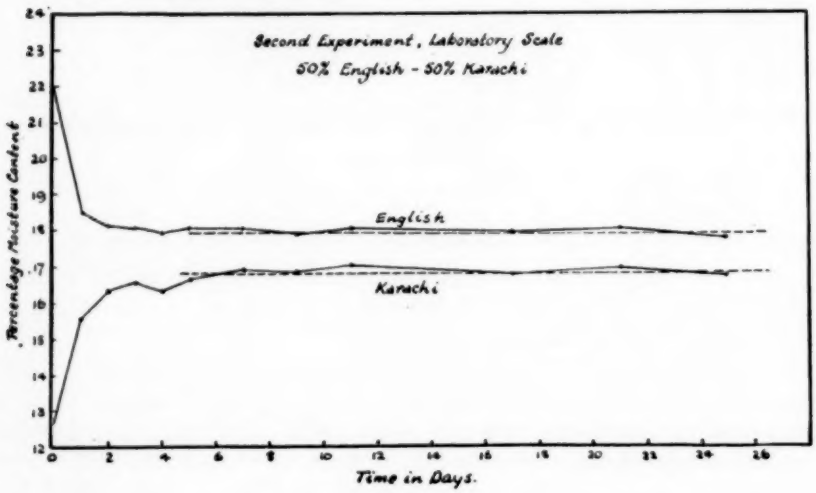

Figure 2

Along with the above a second series of tests was run under similar conditions except that the mixtures consisted of English wheat which had been dried and white Karachi which had been moistened. The wheats were initially the same as those used for the last series of tests,

but several days before the tests the English was dried at 50° C. and the Karachi moistened evenly by spraying. At the time of the tests the moisture contents of the well-mixed wheats were: English, 11.36%; Karachi, 26.56%. The results are given in Table III and Figure 3.

TABLE III

MOISTURE INTERCHANGE IN A 50–50 MIXTURE OF DRY ENGLISH AND DAMP WHITE KARACHI WHEATS

Time in days from start	Moisture of English	Moisture of Karachi	Difference
	%	%	%
0 (before mixing)	11.36	26.56	15.20
1	17.69	21.58	3.89
2	18.93	20.41	1.48
3	19.19	20.14	0.95
4	19.20	19.94	0.74
5	19.25	19.98	0.73
7	19.62	20.08	0.46
9	19.42	20.47	1.05
11	19.47	19.93	0.46
17	19.40	19.83	0.43
21	19.52	20.01	0.49
25	19.29	19.72	0.43
Mean of last 7 determinations	19.42	20.00	0.58

Mean moisture of unmixed wheats, 18.96%.
Mean of means of last 7 determinations, 19.71%.

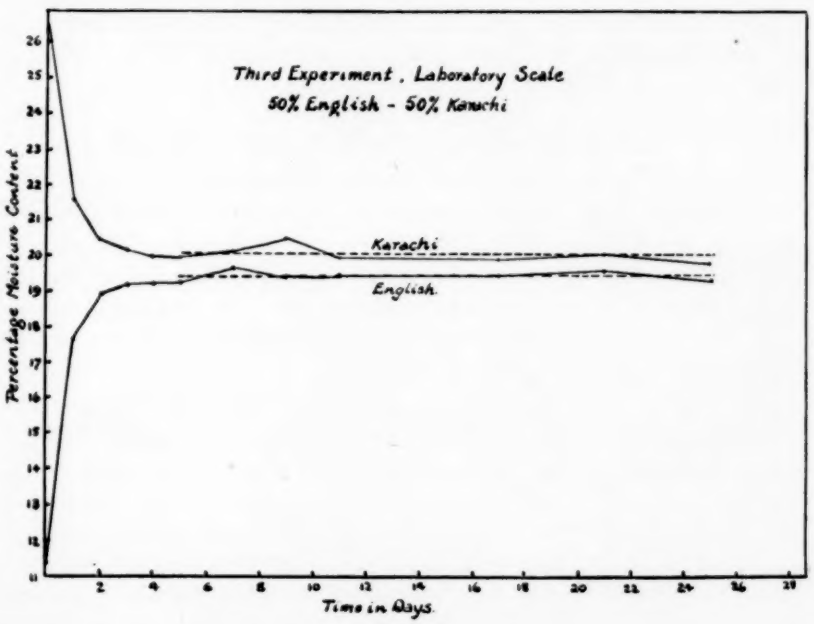

Figure 3

The results recorded are of considerable interest. In all three experiments the change in moisture content was very rapid for the first day and relatively small during the second and third days. After three days no significant change in moisture content occurred. In the large-scale experiment conducted first, the English wheat retained approximately 2.4% more moisture than the Karachi after 45 days; in the second experiment, carried out under more strictly uniform conditions, another and different sample of English wheat retained 1.12% more moisture than Karachi, with no sign of further change during 23 days; in the third experiment, in which Karachi was the damp wheat and English the dry, the Karachi retained 0.58% more moisture than the English, with no sign of change during 22 days. The general conclusions emerge that (1) when wheats of different moisture contents are mixed, a rapid interchange of moisture occurs at first but exact equalisation of moisture content never takes place; (2) however long the mixture is allowed to lie, the damper of the two wheats will permanently remain slightly damper than the originally drier wheat, quite irrespective of whether the damper wheat is soft and starchy, such as English, or hard and vitreous like Indian. This phenomenon is not confined to wheat, but is well known in connection with other colloidal materials. It was observed with silica gel many years ago by van Bemmelen, with wool by W. D. Hartshorne (1918), and with cotton by Orme Masson (1906); while extensive quantitative studies of the phenomenon have been made in connection with silica gel by Zsigmondy, Anderson (1914), and others, and in connection with cotton by Urquhart and Williams (1926).

Rate of Absorption of Water Vapour by Wheat

The point of most interest to millers is the great speed with which the transference of moisture takes place during the first day. Every berry is in actual contact with several other berries at a number of points and some moisture may pass from the damper to the drier berries through these points of contact. It is more probable, however (and there is some evidence to support the view), that the moisture transference takes place via the air. The tables show that the drier wheat gained from 3% to 5% of moisture in the first day and ½% to 1¼% the second, the actual amounts absorbed depending on the initial difference in moisture contents between the two wheats. The change was hardly significant after two days and entirely insignificant after three days. It is interesting that where cold conditioning is the rule, as in U. S. A., the wheat is rarely allowed to lie more than three days, and it is probable that two days are adequate for any wheat. In the light of the above results, it is possible that the well-known

difficulty of getting sufficient moisture into hard, dry wheats such as durum or Indians is due not to the hardness but rather to the dryness of the wheat. Water penetrates a hard wheat quite rapidly, but in raising durums or Indians from, say, 8% or 10% to 16% or 17% moisture it is impossible for the wheat, after whizzing, to retain sufficient moisture in the wet film on the surface to raise the moisture content to the desired amount. Hence the wheat has to be washed and whizzed several times at intervals.

To investigate the matter further, the experiments were repeated with a mixture of 75% English (moisture content 21.39%) and 25% durum (moisture content 9.74%), and moisture determinations were made every two hours for the first 24 hours and then at longer intervals (Table IV and Figure 4). A shorter series was also carried out with a 50–50 mixture of English and durum wheats. All samples were kept in sealed tubes or bottles in a water bath at 20° C.

TABLE IV

MOISTURE INTERCHANGE IN MIXED ENGLISH AND DURUM WHEATS

Time	75% English + 25% durum—moisture content (%)		50% English + 50% durum—moisture content (%)	
	Durum	English	Durum	English
Unmixed wheats at start	9.74	21.39	9.74	21.39
2 hours	13.06	19.95	11.39	18.53
4 hours	13.70	19.58	12.04	18.11
6 hours	14.36	19.15	—	—
8 hours	15.18	19.37	12.98	17.99
10 hours	15.07	19.33	—	—
12 hours	15.23	19.12	—	—
14 hours	15.86	19.14	—	—
16 hours	15.95	19.04	—	—
18 hours	16.17	18.83	—	—
21 hours	16.48	18.90	—	—
24 hours	16.39	18.83	13.97	17.04
2 days	17.23	18.70	14.49	16.86
3 days	17.37	18.80	14.73	16.85
4 days	17.51	18.70	14.61	16.51
6 days	17.49	18.72	14.61	16.50
26 days	17.58	18.64	—	—
29 days	17.55	18.62	14.76	16.51
3½ years	17.65	18.24	14.88	16.08
Mean moisture content after 3 days	17.53	18.67	14.66	16.51
Mean difference after 3 days	1.14%		1.85%	

The rapidity with which moisture was absorbed by the dry durum wheat in the first few hours of the experiment is shown in Table IV and Figure 4. The moisture content of the durum in the 75% English and 25% durum mixture increased from 9.74% to 13.06% in the first

two hours, *i.e.*, an increase of 3.32% on the ordinary air-dry basis. In 8 hours the increase was 5.44% and in 24 hours it was 6.65%.[1] In other words, the amount absorbed in the first two hours was about equal to that absorbed in the next 22 hours. Very little change occurred after two days, and none at all after three days.

In order to compare directly the relative absorbing rates of durum and English wheats, a technique different from that described above was adopted. Circular aluminium pans $2\frac{3}{4}$ inches in diameter by $\frac{1}{2}$ inch deep were used as containers. Sufficient durum or English wheat (about 8 g.) was used to cover the bottom of the pan, *i.e.*, the layer of wheat was one berry deep. The separate wheats were placed

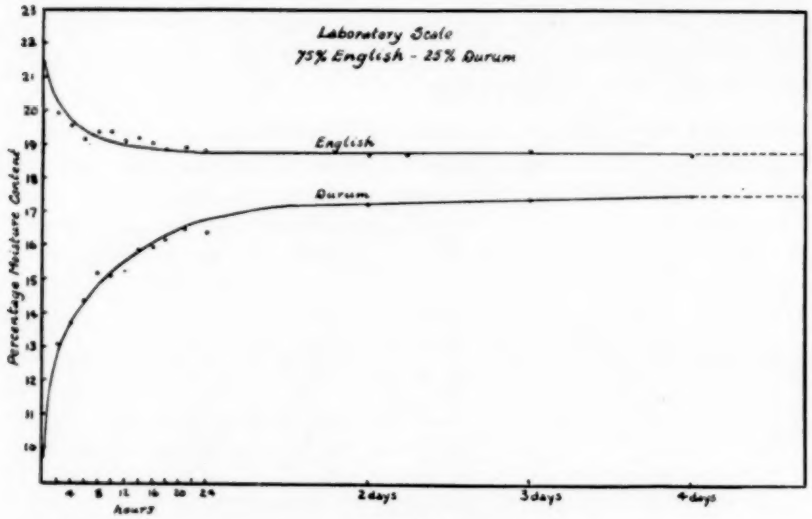

Figure 4

in the pans, which were placed in closed glass vessels of special design which contained 20% sulphuric acid, which is in equilibrium with an atmosphere of 87% relative humidity. The air was not kept mechanically in motion although gentle air mixing must have occurred through convection due to the relatively small temperature differences between the acid and the chamber covers. It is possible that the actual relative humidity near the wheat was lower than that near the acid, and that the "stationary air film" around the wheat was of greater thickness than with wheat in a moving air stream (Lewis, 1922).

Both these factors would lower rate of absorption, and we know from other observations of our own that the rate of absorption under these conditions is actually lower than that observed when wheat of

[1] Calculated as percentages of the dry matter of the wheat, these three figures become 4.22%, 7.10%, and 8.81% respectively.

similar moisture content is treated with an air stream of 87% humidity. The experimental conditions however were such that the acid provided a regulated constant source of supply of water vapour upon which the two samples of wheat could draw quite comparably. The actual rates of absorption are not material to the discussion; only the relative rates under the particular experimental conditions are of interest. The apparatus was so arranged that the aluminium containers with their wheats could be weighed at 15-minute intervals without removing them from their positions, *i.e.*, without interfering in any way with the absorption process. For a detailed description of the apparatus and method of work see E. A. Fisher (1927) and B. A. Keen (1914). The temperature throughout the experiment was 12.5° to 14° C. In these experiments the moisture contents are calculated on the dry weight basis, so that the results are more easily and strictly comparable among themselves. The results are summarized graphically in Figure 5.

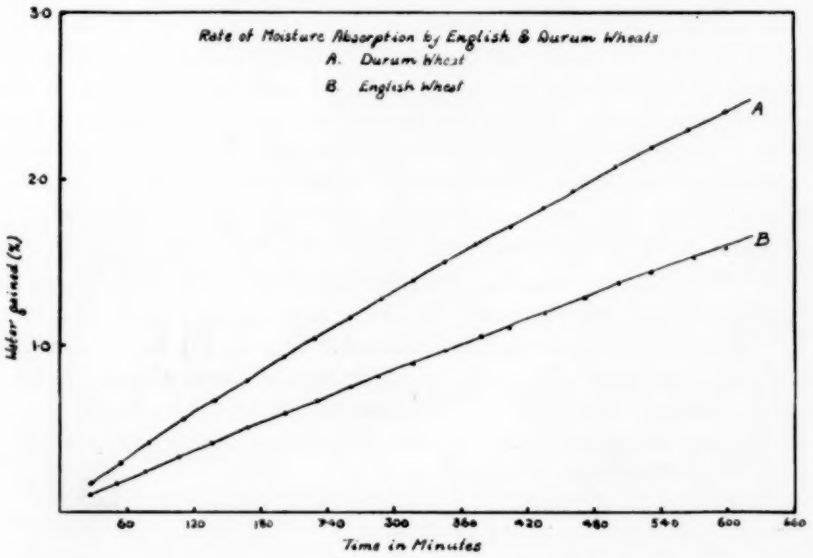

Figure 5

The conditions in this series of experiments were entirely different from those of the earlier series. With the mixed wheats the dry obtained moisture from the damp; the two processes therefore slowed up rapidly as the difference in moisture contents diminished. In these later experiments the wheats were absorbing water vapour from a comparatively large reservoir (the dilute sulphuric acid via the air) of constant vapour pressure. The absorption of moisture therefore proceeded at an almost constant rate; it slowed up very gradually as the

moisture content of the wheat increased. Figure 5 shows that the durum wheat absorbed water vapour approximately 50% faster than the English. The moisture contents, on a dry weight basis, at the commencement were durum 10.79%, English 14.44%; after 10 hours the moisture contents were durum 13.19%, English 16.02%. The average moisture contents throughout the experiment were therefore durum 11.99%, English 15.23%.

It is known that *over a small range*, other conditions being equal, the rate of absorption is approximately inversely proportional to the moisture contents. To make a strict comparison, therefore, of the two rates, the absorption (during 10 hours) of the English (1.578%) should be multiplied by its average moisture content (15.23%) and the product divided by the average moisture content of the durum (11.99%); *i.e.*, $1.578 \times 15.23/11.99 = 2.004\%$, which should equal the absorption (during 10 hours) of the durum wheat. The observed absorption was 2.405%, which was approximately 20% higher than that of the English.

It appears therefore that under strictly comparable conditions of moisture content, atmospheric humidity, and temperature the hard and vitreous durum wheat will absorb water vapour considerably more rapidly than will soft and starchy English wheat.

Two other considerations must be taken into account in a detailed study of this problem: (1) Durum grains are smaller than English; in equal bulks (weights or volumes) of these two wheats there will be more durum grains than English; the durum therefore will possess the larger collecting surface and this factor may be sufficient to account for the observed differences in absorption rates. (2) It has not been demonstrated that water absorbed as *vapour* from the atmosphere passes through the berry at the same rate or in the same manner as liquid water absorbed from a wet film covering the berry such as exists after washing and whizzing.

Summary

It is a common practice to partially dry excessively damp wheat by mixing it with a dry wheat and allowing the mixture to lie for a convenient length of time. It is well known that under such conditions the wheats tend to equalize in moisture content, but little is known concerning the nature and extent of the process of moisture interchange involved. The problem has been studied on both a laboratory scale and a semi-commercial scale and it has been shown that (1) when wheats of different moisture contents are mixed a rapid interchange of moisture occurs at first but exact equalization of moisture content never takes place; (2) however long the mixture is allowed to lie the

damper of the two wheats will permanently remain slightly damper than the originally drier wheat, quite irrespective of whether the damper wheat is soft and starchy, such as English, or hard and vitreous, like Indian or durum.

The speed with which wheat will absorb moisture may be surprisingly great. In a mixture of 75% damp English (moisture 21.39%) and 25% dry durum (moisture 9.74%) the moisture taken up by the durum in the first two hours was almost equal to that absorbed in the next 22 hours. Very little change in moisture content occurs after two days, and the change is entirely insignificant after three days.

The evidence suggests that the well-known difficulty of getting sufficient water into hard, dry wheats such as durums and Indians is due not to the hardness but to the dryness of the wheat. To test this point the rate of absorption of water vapour from the air by durum and by English wheat was measured, and it was found that under strictly similar conditions of temperature, atmospheric humidity, and moisture content, durum wheat may absorb moisture at a rate 20% faster than soft and starchy English wheat.

Acknowledgment

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OBSERVATIONS ON THE RATE OF MOVEMENT OF WATER IN WHEAT

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In an earlier paper (Fisher and Jones, 1939) it was shown that when wheats of different moisture contents are mixed a rapid interchange of moisture occurs. The initial rate of interchange may be surprisingly great if the difference in moisture content is considerable. In a mixture of 75% damp English wheat (moisture content 21.39%) and 25% dry durum (moisture content 9.74%) the moisture taken up by the durum in the first two hours (3.3%) was equal to that absorbed in the next 22 hours. Very little change in moisture content occurred after two days, and the change was entirely insignificant after three days.

The evidence suggested that the well known difficulty of getting sufficient water into hard dry wheats such as durums and Indians is due, not to the hardness, but to the dryness of the wheats. It was found that, under strictly comparable conditions of temperature, atmospheric humidity, and moisture content, durum wheat may absorb moisture at a rate 20% faster than soft and starchy wheat.

It was pointed out that two other considerations must be taken into account in a detailed study of this problem: (1) Durum grains are smaller than English. In equal bulks (weights or volumes) of these two wheats there will be more durum grains than English. The durum, therefore, will possess the larger collecting surface and this factor may be sufficient to account for the observed differences in absorption rates. (2) It has not been demonstrated that water absorbed as *vapour* from the atmosphere passes through the berry at the same rate or in the same manner as liquid water absorbed from a wet film covering the berry such as exists after washing and whizzing.

The whole problem of moisture absorption by wheat is of importance in flour milling technology and further attempts have been made to obtain information that would throw light on the real nature of the process.

One line of study that has been followed is based on the volume changes that must take place when wheat and water are mixed and allowed to remain undisturbed at constant temperature. It is well known that when dry wool, cotton, gelatin, wheat, or flour is mixed with water heat is evolved as indicated by a rise in temperature. The drier the wool or the wheat the greater is the heat evolution.

This heat production is an indication of some kind of union, which may be either chemical or physical, between the solid material and at least some of the water. The phenomenon is quite general. When a sheet of glass is wetted by water some kind of combination occurs between the glass and some of the water as shown by the fact that the glass cannot be *shaken* dry; a film of water sticks to the glass. During the wetting heat is given out, but owing to the small size of the surface the heat production is so small that it can be detected only by means of specially designed apparatus. Very finely powdered glass would show a higher *heat of wetting* per unit weight than sheet glass on account of the greater surface. All colloidal materials show this phenomenon to a relatively great extent on account of their enormous surfaces. It must be pointed out that in this connection the surface of wheat is not merely the outer surface of the berry but, since water can penetrate wheat, it includes also the enormously greater *internal surfaces*. Heat is liberated progressively as water penetrates the wheat berry and so comes in contact with the internal surfaces.

This combination between wheat (and other colloidal materials) and water also results in a contraction in the total volume of the mixture. That is, x cc. of wheat plus y cc. of water when mixed will occupy a volume slightly less than x plus y cc., and this shrinkage will increase progressively until the water has become uniformly distributed throughout the wheat.

Since it is easier to detect and measure small changes in volume than small differences in temperature or in heat production it was thought that the volume-change method might afford a simple means of studying the rate of penetration of wheat by water. If this were the case it should be possible by the same simple means to correlate rate of movement of water in wheat with the original moisture content of the wheat, with type of wheat, with size of berry, possibly with protein content, and even with quality of gluten.

As will be shown later the method has not proved successful owing to certain peculiarities in the structure of the wheat berry, but its study has yielded results of considerable interest and value.

The method of work was extremely simple; the apparatus used is shown in Figure 1. Glass bottles of about 200 cc. capacity were fitted with rubber stoppers carrying lengths of about 30 cm. of fine capillary glass tube. The lower end of the capillary was flush with the bottom of the rubber stopper. A scale, graduated in cm. and mm., was attached to each capillary tube. The bore of each capillary was calibrated with respect to volume in the usual way by means of a mercury column. The capillaries were found to be very uniform in

bore so that the average internal volume per cm. of length could be used without introducing any appreciable error.

Dimensions of three capillaries used were as follows:

	A	B	C
Length of mercury column, *i.e.*, of tube	27.5 cm.	28.3 cm.	27.1 cm.
Weight of mercury column	6.2580 g.	6.3935 g.	6.1900 g.
Density of mercury at 20° C.	13.546	13.546	13.546
Volume of mercury column—weight divided by density	0.4620 cc.	0.4720 cc.	0.4569 cc.
Volume of mercury column divided by length in cm.—vol. per cm. length	0.0168 cc.	0.0167 cc.	0.0169 cc.

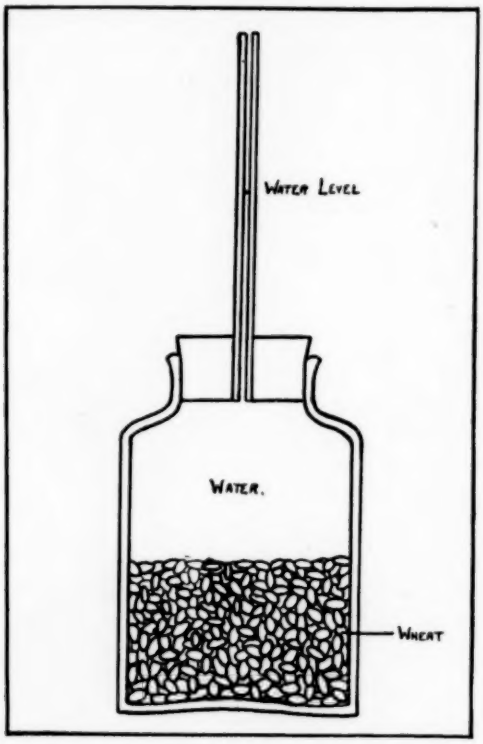

Figure 1

All experiments were carried out in a thermostat at 25° C. The volume changes may be expressed as rise (plus) or fall (minus) in cm. of solution above or below the original height in the capillary. These distances may be converted into volumes (cc.) by multiplying by 0.0168, and these volumes into percentage expansion or contraction of wheat by multiplying by 100 and dividing by the volume of 50 g. of wheat (50 g. of No. 1 Manitoba wheat used occupied a volume measured by displacement of 35.3 cc.). The wheats, the bottles, and

the water or solutions used were kept in the thermostat for at least 12 hours (usually overnight) before use, so that all the materials were at the same temperature, 25° C., before being mixed. Blank experiments were first carried out with water only in the bottles to ascertain whether slight movements of the rubber stoppers were likely to occur during the course of an experiment; such slight movements would cause the water to rise or fall in the capillary tube and so would vitiate the results. Such movements would necessitate the use of ground-glass stoppers. No movements occurred in such blank experiments, indicating that the technique was satisfactory.

The procedure in each experiment was as follows: 50 g. (to the nearest grain of wheat) of hand-picked wheat were placed in a bottle and kept in the thermostat overnight. The wheat was then well covered with distilled water (or solution) which had also been kept in a closed flask in the thermostat overnight, after having been freshly boiled and cooled to drive off dissolved air. The rubber stopper (with capillary tube) was placed in position, the capillary connected by rubber pressure tubing to a filter pump, and the pump turned on for about five minutes, during which time the bottle was occasionally shaken. This procedure removed all air bubbles entangled in the wheat, especially in beards and creases. When no more air bubbles were observable, the pump was disconnected, the bottle filled with water, and the rubber stopper pressed into the bottle sufficiently far for the water to rise to a convenient point near the top of the capillary tube. In every case the first measurement of the height of the water in the capillary was made ten minutes after the first addition of water to the wheat.

In the first experiments with No. 1 N. Manitoba wheat and water an increase in volume occurred during the first 1½ hours, as was indicated by the water rising in the capillary tube, and this was followed by a progressive contraction as shown by the water falling in the capillary. After about twenty-four hours, however, bubbles of gas accumulated in the bottle, the amount of gas increasing with time. In some experiments with English wheat gassing commenced after twelve hours. This gas could only be due to the liberation of absorbed air from the interior of the wheat, to incipient germination of the wheat, or to bacterial or mould action. It is stated in the literature that nitrobenzene solution will inhibit germination and mercuric chloride is a well known bactericide. Two replicate series of four experiments each were carried out with No. 1 N. Manitoba and with English wheat using respectively water, a 0.1% solution of nitrobenzene, a 0.1% solution of mercuric chloride, and a mixed solution of nitrobenzene and mercuric chloride of the same strength. In the

course of a week gas bubbles were produced only in the bottles containing wheat and water, a result which suggests strongly that the gas production observed was due to germination or to bacterial action or to both. In all subsequent experiments a solution of mixed nitrobenzene and mercuric chloride, of a concentration 0.1% in respect of each, was used instead of water alone.

The first wheat studied was No. 1 N. Manitoba of 13.39% moisture content. Duplicate experiments were carried out with water alone and with nitrobenzene-mercuric chloride solution. The results given in Table I show the close concordance between duplicate determinations. A further sample of the same wheat was moistened to 16.08% moisture content and allowed to lie 48 hours before being examined.

TABLE I

VOLUME CHANGES OF MIXTURES OF 50 GRAMS NO. 1 N. MANITOBA WHEAT AND WATER (OR SOLUTION)

(Volume changes are expressed as cm. rise (plus) or fall (minus) of solution in the capillary tube.)

Time after 1st reading	Moisture content 13.39%			
	Water alone		Solution	
Hours	*cm.*	*cm.*	*cm.*	*cm.*
0	0	0	0	0
½	—	—	—	—
¾	—	—	—	—
1	+ 3.35	+ 3.3	+ 2.0	+ 2.2
1½	+ 3.9	+ 3.55	+ 2.45	+ 2.7
1¾	—	—	—	—
2	+ 2.25	+ 2.1	—	—
2½	− 0.10	− 0.10	—	—
2¾	—	—	—	—
3	− 2.4	− 2.55	—	—
3¾	—	—	− 8.5	− 8.1
4	− 6.7	− 6.4	—	—
4½	—	—	—	—
4¾	—	—	−10.8	−10.5
5½	−10.1	− 9.15	—	—
5¾	—	—	−12.9	−12.5
6	—	—	—	—
6½	−11.75	−11.25	—	—
6¾	—	—	−14.9	−14.6
7	—	—	—	—
7¼	—	—	—	—
7½	−12.75	−12.35	—	—
12	—	—	—	—
22¾	—	—	—	—
23½	—	—	—	—
23¾	—	—	−33.7	−33.6
29¾	—	—	−36.0	−35.9
31½	—	—	—	—
47	—	—	−38.5	−38.7
53½	—	—	—	—
71½	—	—	−38.9	−38.9
122	—	—	—	—

Another sample was partially dried in an air oven at 120° C. to 10.87% moisture content. The detailed results are given in Figure 2.

Figure 2 is illuminating. Curves A and B may be regarded as the normal type of curve obtained with colloidal materials in general. The initial rise to a maximum is due to an expansion of the system caused by the heat of wetting, or rather it represents a balance between the expansion due to heat production and the contraction which takes place progressively from the start. The heat produced is slowly dissipated and after some time only the progressive contraction is observed. If the initial moisture content of the wheat is increased,

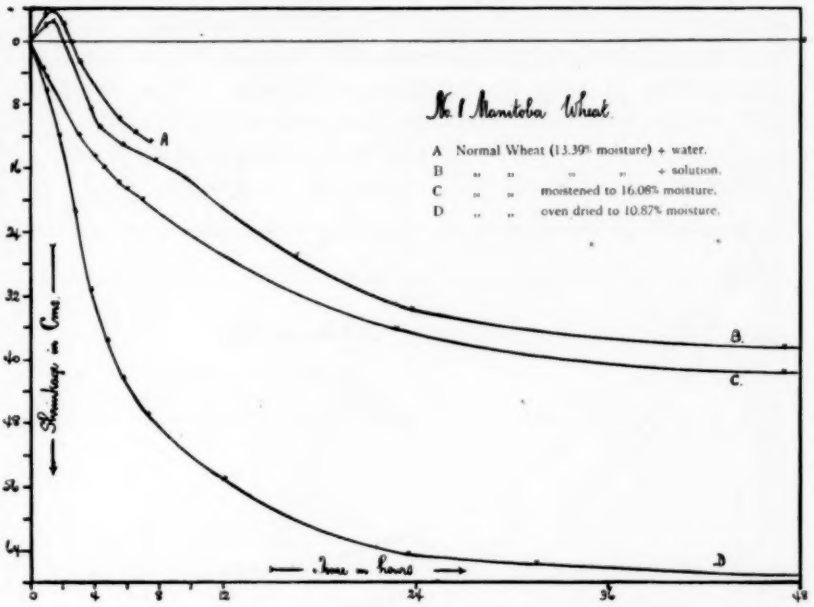

Figure 2

both the initial expansion and subsequent contraction should be reduced. It will be seen from Curve C (Fig. 2) that the initial expansion did not occur when the initial moisture content of the wheat was raised to 16.08% although it was very marked at 13.39%. The contractions were closely similar at both moisture contents. At the same time some heat must have been produced and the disappearance of the initial expansion can only be explained by the production of fine cracks and fissures, or by the increase in size of pre-existing cracks, during the preliminary moistening of the wheat. It is obvious that if such cracks were present or produced, the more or less rapid filling of these would produce a relatively sudden and greatly increased initial

contraction of the volume of the mixture, which would mask completely the initial expansion due to heat production.

This idea is strengthened by Curve D (Fig. 2). During the rapid drying of wheat at 120° C. one would expect fine cracks to develop as they do in timber during too rapid kiln drying. The partially dried wheat showed no initial expansion due to heat production but the contraction was very marked and particularly rapid in the first few hours. The total contraction in 48 hours equalled 1.12 cc. or approximately 3.2% of the total volume of the wheat. The highest total apparent contraction so far recorded with wheats was with a sample of Australian, oven dried to 11.17% moisture content: this showed an apparent contraction in five days of 5.2% of its volume. These values seem altogether too high if the observed contractions are real ones due to imbibition of water by the wheat endosperm. Thus, the phenomenon is more striking with gelatin than with wheat, yet with bone-dry gelatin the total contraction is only 4.0% (approximately) and with a 70% gelatin gel is about 1.7% of the volume of the gel.

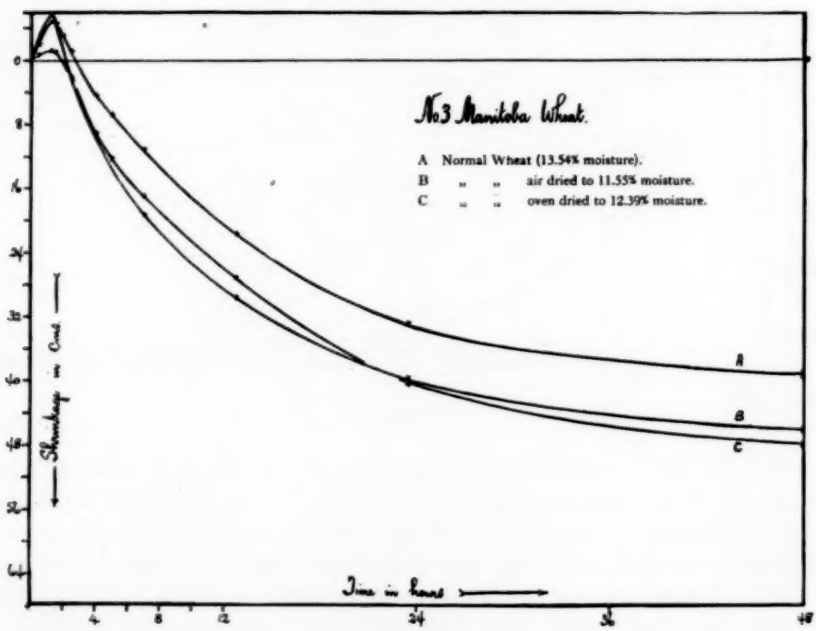

Figure 3

In Figure 3 are given the results obtained with a sample of No. 3 N. Manitoba wheat with an original moisture content of 13.54%. The normal curve (A) is similar in type to that obtained with No. 1 N. Manitoba and shows an initial expansion followed by progressive

contraction. Also, the total contraction in 48 hours was the same in both samples, 38.6 and 39.0 cm. respectively. After removing 2% of moisture by careful air drying at ordinary temperature the initial expansion showed little change while the total contraction in 48 hours was increased from 39.0 to 45.9 cm. On the other hand, removing only half the amount of water by the more drastic process of oven drying almost removed the initial expansion and increased the total contraction (in 48 hours) from 39.0 to 47.6 cm.

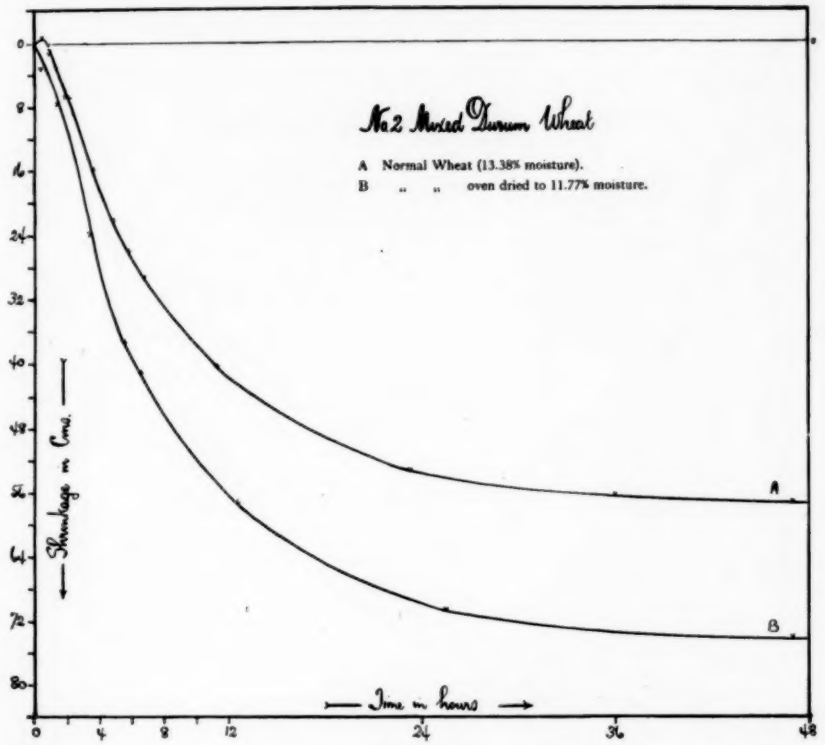

Figure 4

Some results with durum wheat are shown in Figure 4. The normal curve A shows only a slight initial expansion. Removing 1.5% moisture by oven drying obliterated the initial expansion and increased the total contraction from 57.4 to 74.0 cm.

Experiments with Red Standard and Yeoman English wheats are summarised in Figure 5. The normal Red Standard, with an initial moisture content of 15.46% (Curve A), showed a greater and a more prolonged expansion (heat evolution) than any of the other wheats examined: the maximum expansion was 10.3 cm. and no actual con-

traction from the original volume was observed for 5½ hours. Removal of only 0.8% of the moisture by air drying at ordinary temperature reduced the initial expansion and increased markedly the total contraction. The removal of 3¼% of moisture by oven drying obliterated the initial expansion and increased greatly both the total and the rate of contraction. The normal curve for Yeoman wheat (moisture content 14.87%) is also shown (Curve D).

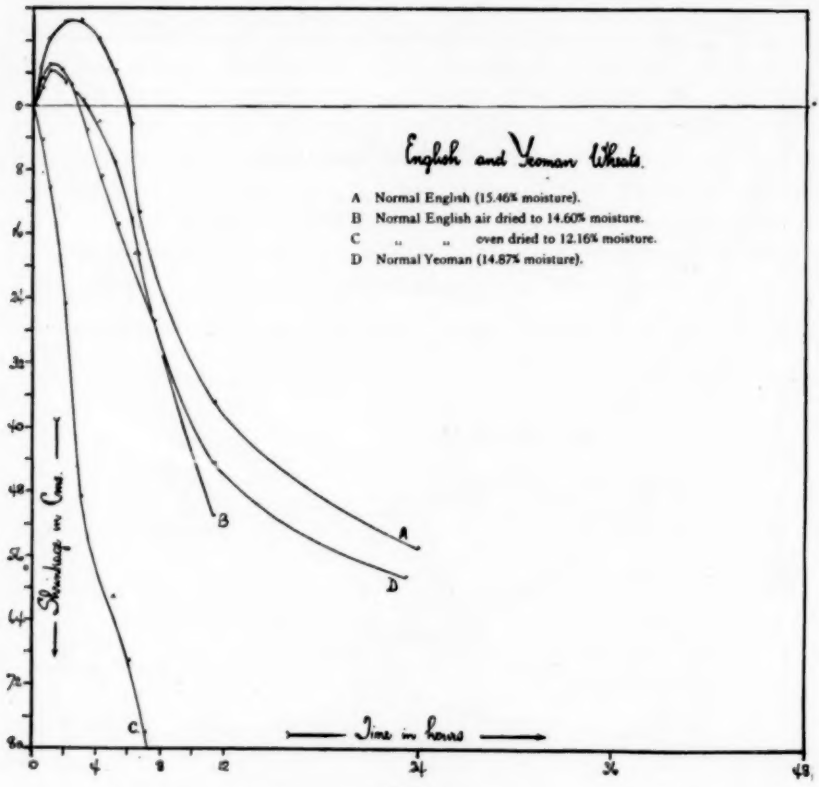

Figure 5

Some results for Plate wheat are given in Figure 6.

A more detailed set of results was obtained with a sample of Australian wheat and these are summarised in Figure 7. The natural wheat, with a moisture content of 15.40%, gave the normal type of curve with initial expansion followed by progressive contraction. Removal of 2.1% of moisture by air drying reduced the initial expansion and increased the total contraction by about 14 cm. Removal of a further 2.1% of moisture had no further effect on the initial expansion but increased the total contraction by a further 24 cm. Oven drying

to the same extents completely removed the initial expansion and increased the total contractions by 51 and 77 cm. respectively (compared with 14 and 38 cm. respectively for the corresponding air-dried samples).

The air-dried sample (moisture content 11.17%) was re-moistened to 15.40% moisture by standing over water in a closed vessel. This procedure removed entirely the initial expansion and slightly increased (by about 2 cm.) the total contraction. In other words, adding

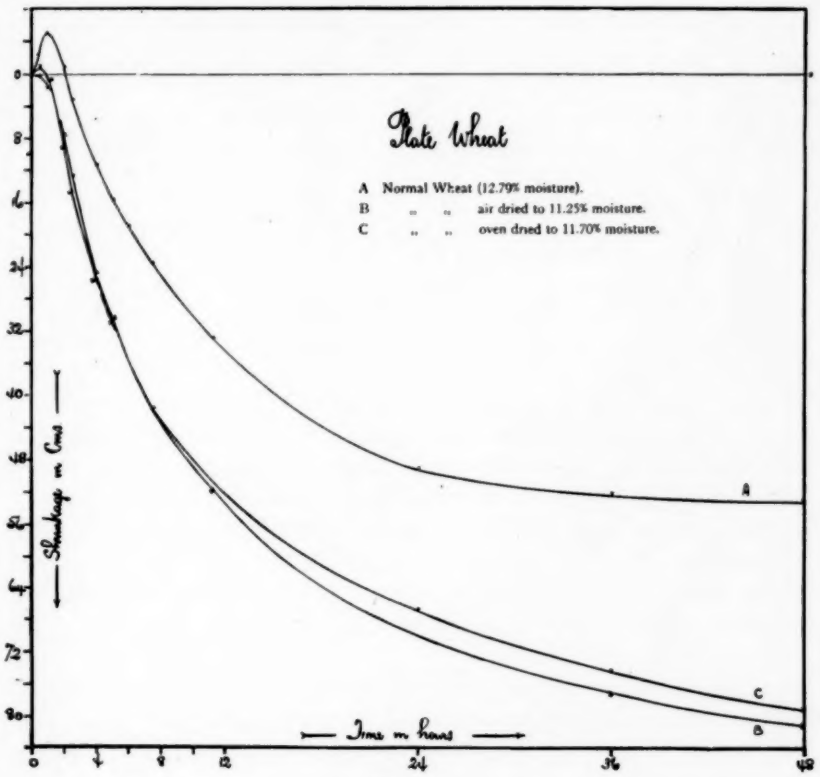

Figure 6

moisture carefully in the form of vapour to the air-dried sample did not cause the wheat to return to its original mechanical condition: Curve F is very different from Curve A.

These apparent anomalies are probably due to the presence of permanent strains set up in the wheat berry as a result of the stresses imposed by the desiccation of the wheat during ripening. These strains would vary with subsequent addition or subtraction of water; actual distortion or even cracking (microscopic) of the wheat would

occur, and the more rapid the removal of water, as in oven drying, the greater the distortion and cracking. In other words, the mechanical and physical properties of a sample of wheat depend to some extent on its "moisture history." Thus it has been shown by Sharp (1927)

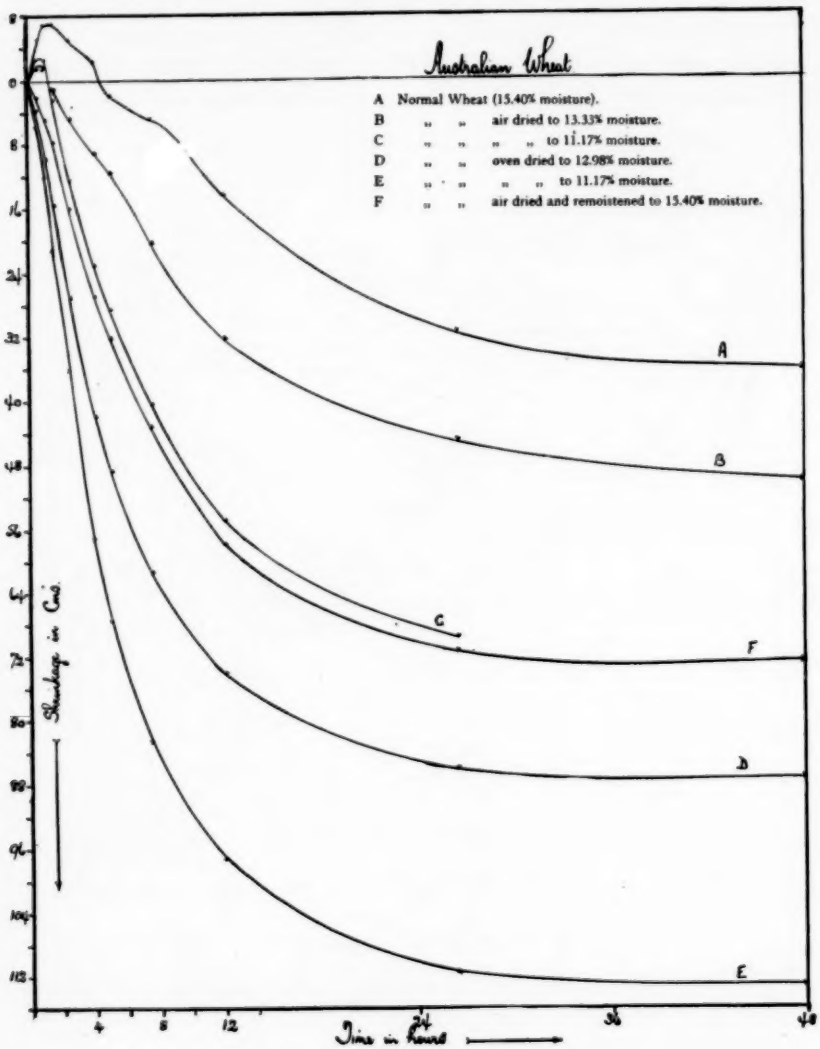

Figure 7

that the apparent density of wheat depends on its "moisture history." If moisture is added to wheat its density is decreased, but if the moistened wheat is carefully re-dried to its original moisture content the original density is not regained; the wheat remains definitely of slightly

lower density. This again falls into line with the earlier work of Thomas (1917), who showed that the bushel weight decreases with increasing moisture content; that is, an increase of 1% moisture means a decrease of approximately ¾ lb. in bushel weight. If the moistened wheat is carefully re-dried to its original moisture content, the original bushel weight is not regained but remains definitely lower. Moreover, this permanent decrease in bushel weight is proportional to the extent to which the moisture content was raised prior to re-drying. Thomas's figures for one sample of spring wheat are given in Table II.

TABLE II

EFFECT OF MOISTURE CONTENT ON THE BUSHEL WEIGHT OF A SAMPLE OF WHEAT (L. M. THOMAS)

Moisture content	Bushel weight	Moisture content after re-drying	Bushel weight after re-drying
%	*lbs.*	%	*lbs.*
11.4	63.0	—	—
13.2	61.0	11.1	62.0
15.2	60.0	11.3	61.5
18.8	56.0	11.0	61.0
22.2	53.0	11.0	60.5
25.2	52.0	11.4	60.0

These results of Thomas have been broadly confirmed in the present writers' laboratory, although the problem appears to be even more complicated than is suggested by Thomas's figures. After wheat is moistened, the bushel weight falls but subsequently rises progressively on standing for several days until a maximum value is obtained; this maximum value is always less, however, than the original value obtained before moistening. This holds up to about 20% moisture content, above which, for various reasons, bushel weight determinations tend to be erratic.

The phenomenon is not without practical significance. It may be a factor in the difference sometimes observed in the bushel weight of a cargo of foreign wheat as recorded in the countries of import and of export respectively, even when difference in moisture content is taken into consideration.

One sample of mixed Nos. 1, 2, and 3 N. Manitoba wheat was investigated at two temperatures, *viz.*, 25° C. (77° F.) and 37.5° C. (99.5° F.). The results are summarised in Figure 8. The rate of shrinkage was at first greater with the warmer sample but the rate diminished earlier with this sample so that at 28 hours the total shrinkage was the same for both. After about 15 to 20 hours the rate of shrinkage became the greater with the colder sample and remained so up to six days, when the experiment was discontinued.

This effect of temperature on rate of apparent absorption of water by wheat is roughly in line with what is known about gelatin and other colloids: the higher the temperature the less water is bound by the colloid and hence the total shrinkage is less. With gelatin, however, the initial rate of shrinkage is also less at higher temperatures, while with wheat the reverse appears to be the case. This reversal is presumably due to greater distortion of wheat at higher temperatures. The total shrinkage must depend on two factors—the filling up of cracks and the real imbibition of water by wheat endosperm. The relative magnitudes of these two factors cannot be assessed, nor is it likely that the ratio of the two factors will remain constant throughout the course of a single experiment.

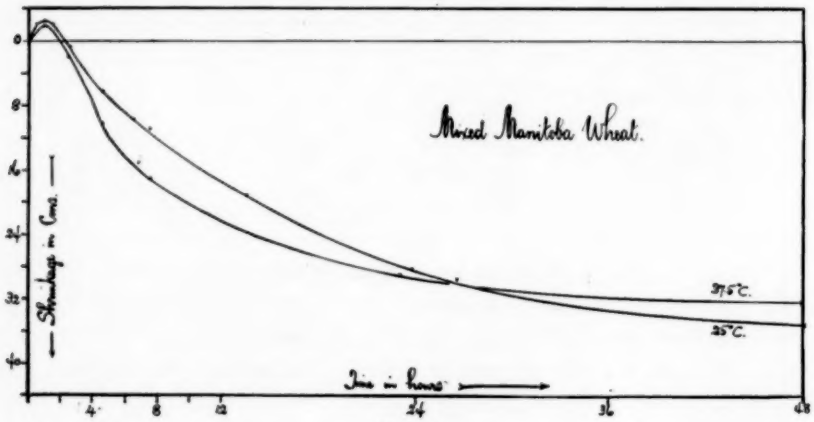

Figure 8

This experiment throws an interesting light on the mechanism of hot conditioning. At the higher temperature wheat will take up moisture more quickly, but this take-up is largely mechanical since actually less water is bound to the endosperm than is the case at lower temperatures. After the hot conditioned wheat has been cooled and is lying in the bin a further proportion of the water inside the wheat becomes bound by the endosperm without further movement.[1] It is possible that this may be an essential part of the tempering process that takes place in wheat when lying after hot conditioning.

As a practical method of investigation the volume-change method has proved somewhat disappointing. The results recorded do, however, throw some light on the mechanical structure of the wheat berry and on the mechanism of wheat conditioning. They also agree with

[1] This, of course, follows from purely thermo-dynamic considerations. Since heat is evolved by absorption of moisture by a colloid, the extent of the absorption must be less the higher the temperature in accordance with the well known Le Chatelier principle. For an interesting discussion of the general problem of the thermo-dynamics of water absorption, see Shorter (1924).

those discussed in an earlier paper (Fisher and Jones, 1939) in showing that very little moisture movement takes place after three days; with the mixed Manitoba sample the total shrinkage during the second three days was less than 3% of that of the first three days, and considerably less than half that of the third day.

Summary

When a colloidal material, such as wool, cotton, gelatin, or wheat, and water are mixed and allowed to stand at constant temperature a progressive shrinkage of the mixture occurs. Thus, if x cc. of wheat and y cc. of water are mixed the volume of the mixture will be slightly less than x plus y cc. and the shrinkage will increase progressively until the water is uniformly distributed throughout the wheat.

An attempt has been made, by a simple but accurate method, to measure these volume changes over periods of days in mixtures of wheat and water. The rates of such volume changes should be related to the rates of movement of water throughout the wheats, to the original moisture content of the wheat, and to other factors. The results obtained, although of considerable interest, are anomalous and can only be interpreted as indicating the presence of fine cracks or fissures in the wheat, or the production of fine cracks or an increase in size of pre-existing cracks during the moistening. Such cracks make it impossible to measure the real shrinkage that undoubtedly occurs.

It is suggested that the cracks may be caused by the presence of permanent strains set up in the wheat berry as a result of the stresses imposed by the desiccation of the grain during ripening. These strains would vary with subsequent addition or subtraction of water, and actual distortion and microscopic cracking of the wheat would result. Such a mechanical condition of wheat would explain various phenomena such as the facts that wheat density and bushel weight are affected by the previous "moisture history" of the wheat.

The effect of temperature is characteristic: a higher temperature appears to hasten materially the movement of water in the earlier stages, *e.g.*, up to 12 hours or so, but to decrease the rate subsequently. The total shrinkage is also less at the higher temperature. This is roughly in line with what is known of other colloids and appears to throw some light on the mechanism of wheat conditioning.

Acknowledgment

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SOME REMARKS ON THE VARYING INFLUENCE OF COMPRESSED YEASTS OF DIFFERENT INDUSTRIAL ORIGIN ON THE GAS RETENTION OF DOUGH, AS RECORDED BY A NEW INSTRUMENT, THE CHEFARO BALANCE [1]

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Cereal chemists, wishing to eliminate the personal factor of the baker's skill in baking experiments, have devoted much time to elaborate experimental methods, whereby one can predict what sort of loaf may be produced with definite ingredients, and it is generally recognized that it is necessary in this endeavor to study a large number of different properties. This, however, requires much time, and therefore during recent years efforts have been made to construct a recording apparatus to produce curves that constitute a basis for evaluating the more important properties of the dough.

Mueller (1937) gives a historical survey of some mechanical methods for the determination of flour and dough properties and mentions among others Boland's "aleurometer" for the determination of gluten quality, constructed in 1880; Liebermann's apparatus for the same purpose; Kosutany's method for the determination of the physical properties of dough, published in 1907, which emphasized the fact that it is much better to study the whole dough rather than the gluten alone; Hankoczy's apparatus, which gives numbers for the quality, the extensibility, and the elasticity of the gluten; Chopin's "extensimeter," constructed independently in 1921, based on the extensibility of a dough; Bühler's "comparator," constructed in 1924 and Barbade's

[1] Paper read before Section VIII on Agricultural and Industrial Microbiology of the Third International Congress for Microbiology, New York City, September 2–9, 1939.

"aleurograph" (1928), both based on the same principle as Chopin's "extensimeter." All these contrivances determine gluten or dough qualities shortly after mixing, and do not permit measurements of dough properties during the rest of the bread-making process.

Mueller (1937) further describes mechanical devices based on the force required for mixing the dough; he mentions Hogarth, Hankoczy, Deutschrenner, Bailey, and Brabender's farinograph. Although no effort is made here to present a complete review, mention may further be made of Swanson and Working's recording dough mixer; a Belgian apparatus called Varmi's "patograph," based on the same principle as Brabender's farinograph; Chopin's recording mixing apparatus, which delivers mechanically the dough disks required for his "alveograph," which is an improved form of his "extensimeter."

Gas production during the fermentation of dough is an extremely important factor and some methods for its determination are described by Elion (1933). Recording apparatuses for gas production are, for example, Brabender's "fermentograph" and some recent Belgian contrivances, such as Varmi's "volumetrograph," which records the total gas production and the volume obtained by the fermenting dough in a cylinder, and Varmi's "panigazograph," which measures the gas produced during a baking process.

For the purpose of determining gas retention by the dough during fermentation and the factors influencing it, no recording apparatus has been available which would enable one to follow the gas retention *during the whole period of the fermentation* instead of only during some short periods thereof. It is not sufficient merely to know the quality at the beginning of the fermentation, at some definite moment during the fermentation, or after the fermentation (finished loaf). The influence of diastatic and proteolytic enzymes is so important that it is desirable to record during the whole period of fermentation.

This requirement has been met by the Chefaro balance, developed and patented by the Chemische Fabriek Rotterdam, Rotterdam, Holland. Before discussing the experiments made with this apparatus, a description will be given.

The Recording Chefaro Balance

The Chefaro balance (Fig. 1) is an analytical precision balance, by which the quantities of gas retained and produced by the fermenting dough can be accurately weighed and automatically recorded. In a special gas-jar (Fig. 2), which is open at the bottom, a dough ball is placed on a small scale and the gas-jar is attached to one beam of a balance, after being immersed in a water bath which is electrically heated and kept automatically at the desired temperature. A special

construction allows the liquid of the bath to rise into the gas-jar until a definite point under the scale is reached. The other beam of the balance bears a counter-weight which can be moved and has at its end a special pen. The recording drum is rotated by a clock and moved on and back again by an electric motor; the pen writes 30 points per minute in an unbroken line on the graphs. This system eliminates any friction during recording.

Each apparatus consists of two complete balances, the two special gas-jars being immersed in the same water bath.

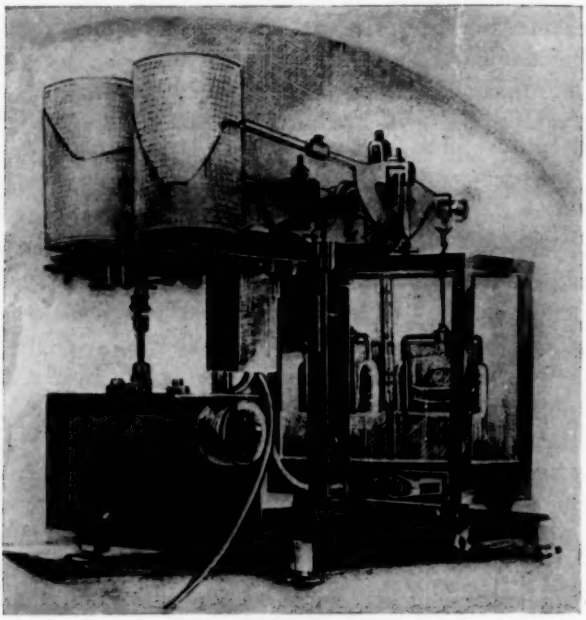

Fig. 1. The Chefaro balance.

The gas produced by the fermenting dough displaces liquid from the gas-jar, which therefore becomes lighter and rises in the water bath; the writing pen at the end of the balance beam descends accordingly and the gas production is recorded. It is necessary of course that the gas produced be insoluble in the liquid of the bath, for which purpose the manufacturers supply a liquid to be mixed with the water in the water bath.

If gas retention is to be recorded, ordinary water can be used in the water bath and on the lower compartment of the small scale in the gas-jar a solid material is placed, which absorbs the carbonic acid escaping from the dough, so that only the increase of the dough volume, *i.e.* gas retention by the dough during the whole fermentation period, is recorded.

The manufacturers give full details as to the manufacture of the small doughs required for the experiments. Water absorption of each flour is determined with a centrifuge and the percentage of water found in this way is used in the making of the small dough. Each flour therefore obtains its own quantity of water and for the purpose of obtaining comparable results, the doughs placed on the scales are weighed so as to contain each the same quantity of flour, three grams of flour being used for the determination of gas retention. If one would use doughs of the same total weight, the stronger flour would be at a disadvantage as compared with weaker flour, since a dough made from the latter with the same weight of dough would contain less water and more flour

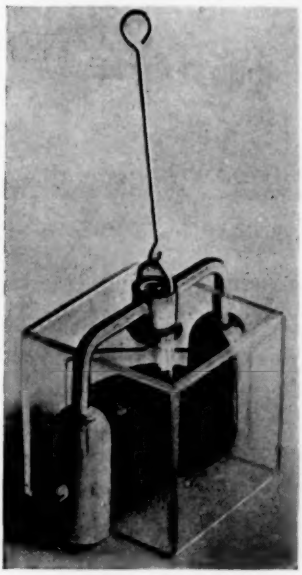

Fig. 2. The gas-jar.

and yeast than a dough made from a stronger flour. To compensate for the small volume of the dough, extra yeast and sugar are employed. When determining total gas production, even smaller doughs can be used, containing one gram of flour only.

Significance of the Curves

The curves in Figure 3 show the following factors: (*a*) the gas-retaining capacity or the highest gas-pressure the dough can support, expressed in cubic centimeters expansion of the dough ball, (*b*) time required until the full development of the dough, and (*c*) the stability of the dough after reaching full development. Flour sample *I* has a slow fermentation; the dough can tolerate only a very slight expansion

and the stability is also inferior. Sample *II* ferments much more quickly; the gas-retaining capacity and the stability are much greater. Sample *III* has a somewhat slower fermentation, but the gas retention and the stability of the dough are the best of the three.

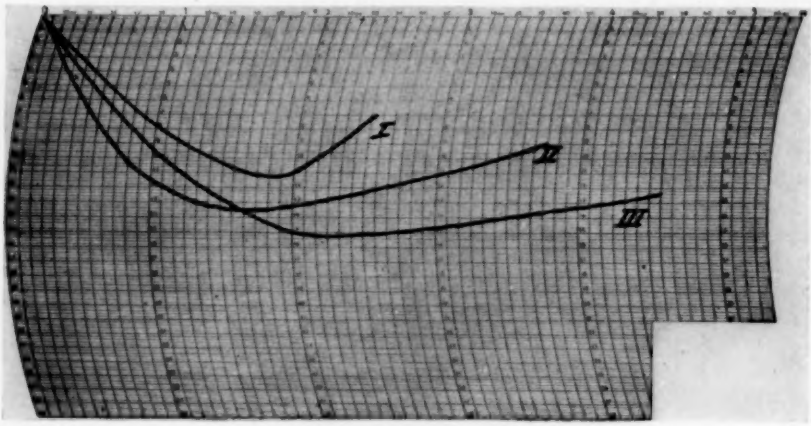

Fig. 3. Curves showing different dough properties.

Influence of Flour Improvers

The Chefaro balance is especially suitable for showing the influence of different flour improvers on the baking quality of flour samples, and for indicating the quantity of improver which gives the best results, since gas retention during the whole period of the fermentation is recorded. Improvers may change the curves considerably, and overtreatment may easily be demonstrated by means of the gas-retention curves.

Influence of Different Yeasts on the Gas Retention of Dough

As the Chefaro balance records gas production and gas retention of dough during the whole period of fermentation, it is especially adaptable for a study of the influence of compressed yeasts of different industrial origin on the gas retention.

It may be expected that gas retention depends on gas production, since lack of gas production will prevent a dough with good gluten qualities from reaching the maximum volume consistent with the gas-retention capacity of the dough. For the purpose of the present experiments it would therefore be desirable to deal with different yeasts which give approximately the same gas production in doughs made from a definite flour sample, and this has indeed been possible.

As a suitable flour for this purpose a Netherlands inland flour was chosen, which had a water absorption capacity of 58.3%. According

to the instructions of the manufacturers of the Chefaro balance, the doughs were made of 5 g. of flour, with addition of 4% yeast, 4% glucose, 2% NaCl, and the necessary water according to an absorption of 58.3%. From one dough were weighed: (1) a dough containing 3 g. of flour for the determination of gas retention and (2) a dough containing 1 g. of flour for the determination of total gas production. Both determinations were made at the same time in one water bath, which was kept at a temperature of 35° C. In this way a large number of experiments have been made with the same flour and different compressed yeasts, originating from different European countries and kindly placed at our disposal by a number of yeast manufacturers.

It is desirable in these experiments to compare yeast samples of the same freshness, and to perform the tests under the same conditions. Under these circumstances duplicate determinations give curves which correspond very well. This is illustrated by the curves of Figure 4, which have been taken by way of example. Curves *I* and *II* are duplicate gas-production curves and *III* and *IV* duplicate gas-retention curves.

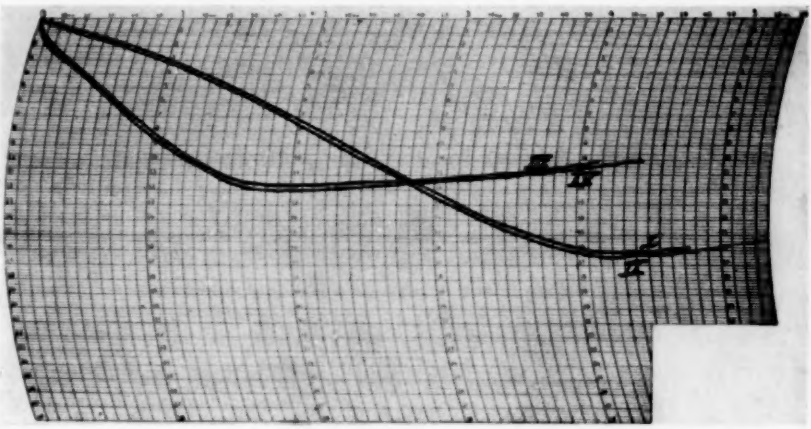

Fig. 4. Agreement of duplicate tests for gas production and gas retention.

Figure 5 gives by way of example the results obtained with four different yeasts. The four gas-production curves are indicated with *I* and the four gas-retention curves with *II*.

It may be concluded that the gas-production curves are similar in character, although the absolute value for gas production in one case after 3 hours differs from the others. The gas-retention curves, however, show some remarkable differences. In the first place two curves only (broken lines) have a regular course comparable to curves *III* and *IV* of Figure 4, but the two others (full lines) show after about 50

minutes an interruption of the regular course. This indicates a sudden escape of gas from the dough and a corresponding decrease of its volume, after which the further gas production causes a new increase of the dough volume. At the moment of the sudden escape of gas, the dough was obviously incapable of retaining all the gas, probably because there was at that moment no good proportion between gas production and gas retention capacity and as a consequence the maximum volume reached by the dough is smaller than in the case where a sudden escape of gas did not occur. Furthermore the gas-retention curves of the different yeasts during the second and third hour show relatively great differences in gas volume retained by the dough, although the gas production curves do not show such important differences in the corresponding periods.

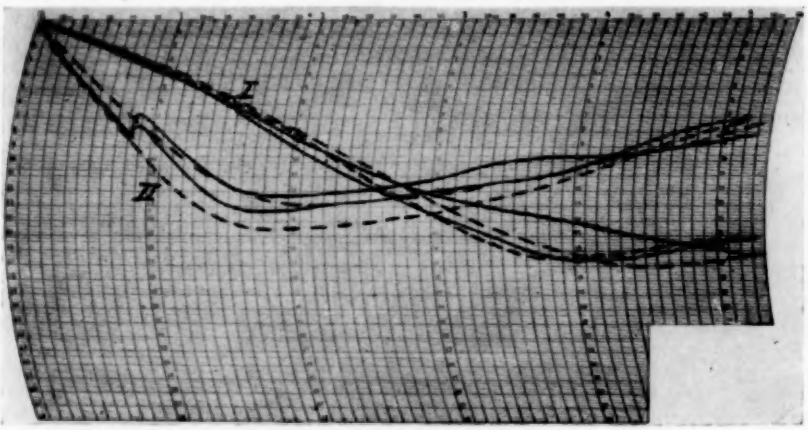

Fig. 5. Variations in gas production and gas retention with different yeasts.

This indicates that the four yeasts have a different influence on the gas-retention capacity of the dough made from the same flour under the conditions of these experiments, a difference which is not caused by differences in gas production.

According to the gas-retention curves obtained, the yeasts can be classed into two groups: those giving a sudden escape of gas (full lines) and those not giving such an escape of gas (broken lines). It is very interesting to note that the yeasts which caused a sudden escape of gas were yeasts made from molasses mashes, while the yeasts which did not cause such an escape of gas were made from grain mashes. We have studied a larger number of yeasts, made of grain mashes as well as of molasses mashes, and all the yeasts have given the same kind of gas-retention curves; *i.e.*, all the molasses yeasts caused a sudden escape of gas from the dough after approximately 50 minutes, while

all the grain yeasts did not cause any sudden escape of carbonic acid from the dough.

This particular difference between the two kinds of yeasts seems to be somewhat related to flour quality, as shown by some preliminary experiments with other flours. It may not be as easily demonstrable with all flours, but the time available for submitting the abstract of this paper to the Congress has made it impossible to investigate this question. The matter will be studied at a later date. For the same reason it has been impossible to study thoroughly the question as to why yeasts made from a molasses mash have a different influence from that of the grain mash yeasts on the gas retention of dough made from the flour represented in Figure 5. We intend to study this question further, also, but some observations may be given here.

Gluten quality has a great influence on gas retention of dough. Flour contains proteolytic enzymes, which can attack the flour proteins and may therefore have an unfavorable influence on gluten quality. The possibility arises that the varying influence of different yeasts on gas retention has something to do with proteolytic activity in the dough.

It is generally admitted, however, that living yeast persistently retains its proteinase. According to R. Willstätter and W. Grassmann (1926), living yeast cells which are not damaged do not secrete proteinases. According to W. Grassmann and H. Dyckerhoff (1928) and W. Grassmann, O. v. Schoenebeck and H. Eibeler (1931) yeast, even when autolysed in the presence of cell poisons, secretes only very insignificant quantities of proteinases during the first hours; only after a duration of the autolysis for about 15 hours does the secretion of proteinases begin.

As far as the author knows, no experiments have been published which demonstrate the secretion of proteinases by living, undamaged yeast. Some papers dealing with yeast proteinase (R. Geoffroy and G. Labour, 1934; A. V. Blagoveschenski and M. P. Yurgenson, 1935) describe only experiments in which the yeast has been treated in the presence of cell poisons, such as toluene.

On the other hand, until some years ago it has been believed that flour contains only small quantities of proteinases. H. Jørgensen (1935, 1935a) developed a very interesting theory on the nature of the action of chemical flour improvers, such as potassium bromate. According to this theory $KBrO_3$ and other flour improvers of its kind paralyze (more or less completely) the proteinases of the wheat flour. Under these circumstances the break-down of the proteins of the dough diminishes and, as a result, the gas-retaining capacity of the dough and the baking strength increase.

In a further paper relative to this subject, H. Jørgensen (1935b) concludes that the reduction in solubility of flour nitrogen that is produced by $KBrO_3$ in flour-water suspensions is strongly increased by the addition of yeast. He explains this by assuming that the addition of yeast causes an increased activity of those proteinases in the suspension which can be inhibited by $KBrO_3$. He further concludes that this increased activity is not caused by proteinases originating from the yeast, but that the flour proteinases are activated by the presence of yeast. To prove the correctness of this conception, Jørgensen describes experiments with flour the proteinases of which have been destroyed or considerably reduced in activity by heating the flour for 12 hours at 95° C. Suspensions of such flour in water, to which yeast was added, gave no increase of the activity of the proteinases in question, while the proteins in the flour could still be attacked by other proteinases of the papain type.

These results of Jørgensen were obtained with dried yeast as well as with fresh yeast. The author states that in the case of dried yeast the activation of the proteinases in question may be caused by the fact that dried yeast secretes glutathione and that this glutathione activates the flour proteinases susceptible to $KBrO_3$ in the same way as is done by Ambros and Harteneck's phytokinase (1928, 1929). Jørgensen demonstrates that glutathione may readily be extracted from dried yeast, the extract giving a positive reaction with sodium nitroprusside.

Although this theory on the effect of yeast on flour proteinases is very attractive, it does not explain how living yeast, which has not been damaged in any way, can activate the flour proteinases, since living yeast does not secrete glutathione, as may be demonstrated by the negative reaction with sodium nitroprusside.

In another paper H. Jørgensen (1936) shows further evidence of the existence of powerful but latent proteolytic enzymes in wheat flour, which can be stimulated by activators such as glutathione or yeast-water.

V. Carbonnelle (1938) determined the quantity of glutathione present in two yeasts made from grain mashes and in one yeast made from a molasses mash. He found that the quantity of glutathione in the molasses yeast was about 60% only of the quantity present in the grain yeasts. If indeed the glutathione of living yeast would exert in an unknown way any activating effect on the flour proteinases, *i.e.* a softening effect on the gluten, the curves found in our experiments for the gas retention of the doughs (Fig. 5) would be easily explained. The grain yeasts, containing more glutathione than the molasses yeasts, would according to Carbonnelle, weaken the gluten of the flour

more than would be done by the molasses yeasts. The fact that the doughs made with molasses yeasts (full lines in Fig. 5) showed a sudden escape of gas proves that at that moment the doughs were stiff and unable to retain the gas, whereas the doughs made from the grain yeasts became somewhat weaker by increased proteinase activity and consequently were able to retain the gas.

Although the results of our experiments would find an explanation in this way, it must be stated once more that further experimental evidence as to the influence of glutathione on flour proteinases in the case of living, undamaged yeast is needed.

Some remarks, however, may be made in this respect. W. Grassmann and H. Dyckerhoff (1928) discuss the activated and the non-activated forms of proteinases present in papain and in yeast. They draw attention to a distinct difference between these enzymes. The non-activated papain *requires* the presence of activating substances like hydrocyanic acid or Ambros and Harteneck's phytokinase for the hydrolysis of peptones, but for the splitting of proteins these activators are useful but *not necessary*. In the case of yeast proteinase, on the contrary, the addition of a biological activator or of an activator foreign to the cells is *necessary* for splitting proteins and *not necessary* for the hydrolysis of peptones.

A. K. Balls and W. S. Hale (1936, 1936a, 1938) demonstrated that flour proteinases are of the papain type.

In view of these observations, one might suppose that flour contains a biological activator which would enable the break-down of proteins by yeast proteinases. In experiments with heated flour Jørgensen (1935b) shows that the proteins in heated flour are attacked by papain but not by yeast, and he considers this fact as a support of his theory that yeast activates flour proteinases but does not secrete proteinases itself. As a result of heating the flour, its proteinases are destroyed or considerably reduced in activity, and it may be possible that this will also be the case with any biological activator of yeast proteinases, if present in the flour. Since papain, according to Grassmann and Dyckerhoff, should be able to attack proteins without addition of an activator, while yeast would *require* such an activator, the experiment of Jørgensen with heated flour-water suspensions and yeast is not quite convincing. In the case of the attack on the proteins of heated flour by yeast the addition of an activator, foreign to the yeast cell, might be required, and as heating the flour might have destroyed such an activator in the flour, if present, it would be necessary to add an activator to the experiment with heated flour and yeast. Perhaps glutathione might be able to serve as such an activator, since according

to Jørgensen glutathione alone is unable to attack the proteins of heated flour.

Jørgensen's theory on the nature of bromate action has found much approval among cereal chemists, but also some objections have been raised. Jørgensen (1938) discusses these publications and shows why some of the objections raised are wrong. He has not yet had the opportunity of discussing the objections raised by W. P. Ford and A. M. Maiden (1938). These authors added glutathione (GSH) to the dough and found with Brabender's farinograph that an addition of 0.005% GSH to the flour weakened the dough considerably in 10 minutes. Repeating the experiments with papain, they found that an addition of 0.03% of papain gave approximately the same curve in 10 minutes and they concluded that 0.03% papain would be equal to 0.005% GSH. These authors then left the doughs for two hours and found that after this time the papain doughs were much weaker than the GSH doughs. Ford and Maiden believe that *if* GSH really acts by stimulating the flour proteinases, the quantities of GSH which after 10 minutes gave the same results as did a definite quantity of papain should also duplicate the results with papain after two hours. Since this is not the case, Ford and Maiden conclude that GSH does not act on the consistency of the dough by stimulating the flour proteinases, but by a direct action on the proteins of the flour. They further state that if Jørgensen's theory of the action of glutathione is wrong, there is reason to doubt also his theory of the action of bromate.

Jørgensen (1938) states that according to a private communication received in 1937 from J. T. Flohil, Minneapolis, the latter made an observation similar to that of Ford and Maiden, but instead of glutathione he used wheat germ, which however contains glutathione, according to Sullivan, Howe and Schmalz (1936).

We do not have Flohil's communication. When considering the normal farinograph curves published by Ford and Maiden (1938) for a dough containing 0.005% glutathione or 0.03% papain, some doubt may arise as to whether these quantities are really to be considered as having the same effect on the dough during the first period after mixing, since the curves are not exactly the same. If this doubt would prove to be correct, one may not expect that these quantities will have the same effect after a period of two hours. Furthermore, in view of our observations mentioned above, glutathione would only be an *activator* of proteinases, while papain would be a complete *proteolytic enzyme* which does not require any activator for the break-down of flour proteins. If Ford and Maiden in one experiment add only 0.005% of a proteinase *activator* (GSH) and in the other experiment much more (0.03%) of a fresh *proteolytic enzyme*, one may hardly expect that

in the long run the small quantity of proteinase activator would be able to equal a much larger quantity of proteinase added (which may moreover itself contain an activator), even when the chosen quantities would give similar results on the farinograph during the first period after mixing.

A similar situation will perhaps apply to Flohil's experiments, since he used wheat germ, which contains glutathione.

The author hopes to be in a position to present further experimental evidence on this interesting subject in the future.

Summary

The author describes a new apparatus, the recording Chefaro balance for the determination of gas retention and gas production during the whole period of fermentation of dough, which has been developed and patented by the Chemische Fabriek Rotterdam, Rotterdam, Holland. He furthermore describes his experiments with this apparatus, from which it appears that compressed yeasts of different industrial origin have a varying influence on the gas-retention capacity of dough. The author discusses some theories which might help to explain the differences found.

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A COMPARISON OF THE ALLIS-CHALMERS AND THE BUHLER AUTOMATIC EXPERIMENTAL MILLS [1]

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(Received for publication May 22, 1939)

The production of flour by means of the experimental mill has always been a problem to cereal chemists because of the time-consuming nature of the procedure, the difficulty of training men to do the work, and the rather wide range of replication errors. There is no doubt that any automatic experimental mill capable of producing reasonably good flour would be very much welcomed by all cereal technologists who have to test wheat samples for baking quality.

Müller (1934) described an automatic mill made by the Brabender Company in which were used two pairs of stone-type grinders. The flow was simple and rapid. The mill was designed primarily to produce flour for the farinograph. Geddes and Aitken (1937) made a comprehensive comparison of flours produced on this mill and on the Allis-Chalmers mill and concluded that the flour from the Brabender mill is not suitable for use in differentiating hard Canadian wheats, although it gave fairly good results with softer types of wheat. There seems to be no record of any work on hard winter wheats with this mill.

In 1935 the Buhler Company of Uzwil, Switzerland, designed and built an automatic experimental mill which embodied many desirable

[1] Contribution No. 60 of the Department of Milling Industry, Kansas Agricultural Experiment Station, in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the state agricultural experiment stations of the hard winter wheat region.

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features. It has a three-break, three-reduction flow, which, although longer than the Brabender, is considerably shorter than the customary procedure with the Allis mill. A good description of this mill is given by Ziegler (1938).

A somewhat detailed discussion of the flow of the Buhler mill is given by Anderson (1938) in a comparison of commercially milled flours and flours milled by the Buhler mill, both from the same wheat mix. It was found that the flours from the Buhler mill were lower in diastatic activity, higher in ash, and about the same in protein content. The time required for the Buhler mill was materially less than for the Allis-Chalmers experimental mill. However, he concluded that the Buhler mill, although automatic in operation and amenable to very close adjustment, could not be operated to advantage by a novice, but required the supervision of a person having a good technical knowledge of milling.

The automatic features of this mill, and the nature of the flow employed in it, created so much interest that it was thought advisable to make some direct comparisons of the Buhler with the Allis-Chalmers mill, with which most cereal chemists are familiar. This should permit a useful appraisal of the performance of the Buhler mill. In this first comparison no attempt was made to compare flours with respect to baking quality, as was done in the work of Geddes and Aitken (1937). The chief purpose was to get an estimate of the degree of reproducibility of milling results.

Accordingly only one lot of wheat was used, and the flour samples were merely analyzed for moisture, ash, protein, and diastatic activity. The millings were made simultaneously on the Buhler and Allis-Chalmers mills, both of which were located in the same room. The work was performed by two millers experienced in the operation of both mills. The test was continued over a period of four days, the first two and the last two being consecutive. The millers alternated on the mills by days, so that each miller had two full days' work on each mill. No attempt was made to control the atmospheric conditions of the room during this test. The wheat milled during the first two days was tempered to 16% moisture in the usual manner. This moisture content, however, proved to be somewhat high for the Buhler mill, and for this reason the moisture was dropped to 15% for the last two days of milling, which was above the optimum for the Buhler but was a little too dry for the best operation of the Allis mill. Thus the wheat was closer to the optimum for the Allis mill on the first two days and closer to the optimum for the Buhler mill during the last two days. The analyses, however, show no significant differences that might be attributable to this procedure.

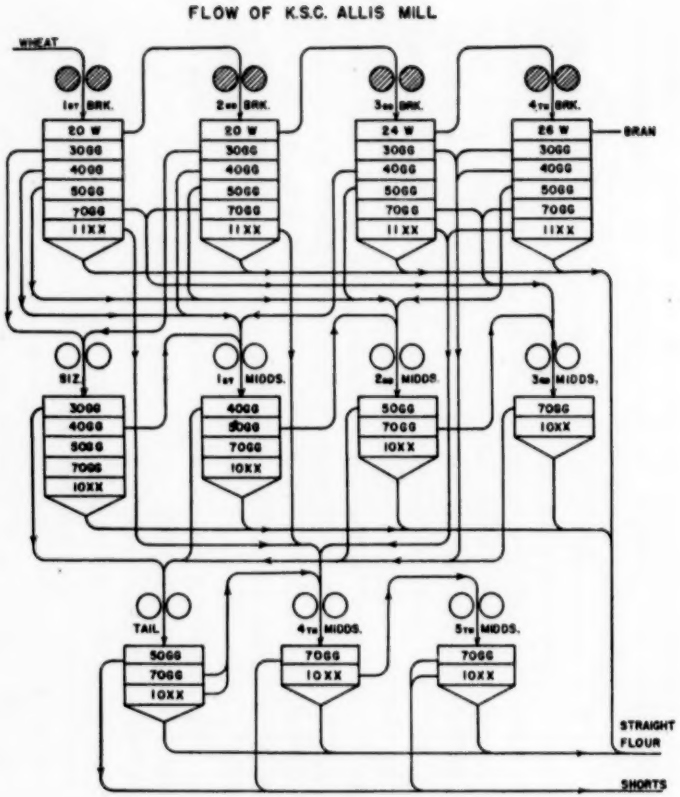

Fig. 1. Flow sheet of the Allis-Chalmers mill.

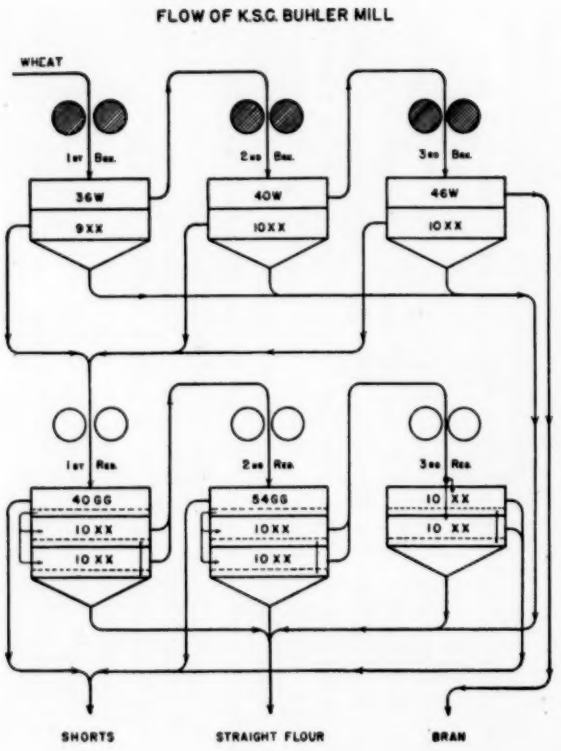

Fig. 2. Flow sheet of the Buhler mill.

As it was desired that a full day's milling be made on each mill each day, the number of samples had to be different because it required the same amount of time to mill seven 2,000-gram samples on the Allis mill as eleven on the Buhler mill. Therefore the comparisons for each day's millings are on the basis of these numbers of samples.

For the benefit of those not familiar with the Buhler mill and because the flow used on the Allis mill may be somewhat different from that used by some workers, the flow sheets of these mills have been shown in Figures 1 and 2.

The Buhler mill, because of its finer adjustments and continuous flow, does not have the errors due to handling of stock that are found in using the Allis mill. The Allis mill, however, has the advantage of the longer and more flexible flow, thus enabling the operator to correct for mistakes made during the milling process. In other words, the errors with the Buhler mill are errors due to the very short flow, where the breaking must be more severe and the grinding harder, which results in a higher-ash, shorter-extraction flour, while the errors with the Allis mill are caused by the handling of the various stocks, necessitated by the discontinuous flow.

The data were analyzed by Fisher's methods and examined particularly for evidences of differentiations between (1) mills, (2) millers, and (3) days.

Flour Yields, Ash, Protein, and Diastatic Activity

For convenience, the analytical results and the yields of the flours have been presented together in Table I.

The flour yield by the Buhler mill was consistently slightly lower than by the Allis mill. While the difference was significant on only two of the four days, there is little doubt that the Buhler mill because of its short system naturally tends to produce somewhat lower yields of flour than the longer-flow Allis mill. It is interesting to note that despite the lower yield, the ash of the flour is significantly higher.

The protein of the flour was higher in the case of the Buhler in all instances, but the differences were found to be significant only for the first two days' millings. The diastatic activity of the Buhler flours was significantly higher in all instances than that of flours milled on the Allis mill.

In this comparison the variability was greater for the Buhler mill in nearly all instances. From this investigation it may be concluded therefore that experienced millers can replicate milling better on the Allis than on the Buhler mill. The data show no differentiation between the millers, indicating either that they both possess the same skill or that neither mill is likely to differ much in variability of

TABLE I

MEANS AND STANDARD ERRORS OF YIELDS, ASH, PROTEIN AND DIASTATIC ACTIVITY OF ALLIS-CHALMERS AND BUHLER FLOURS

		Allis-Chalmers		Buhler	
	Day milled	Mean	Standard error	Mean	Standard error
Flour Yield Percentage (15% moisture basis)	I	72.9	0.87	72.8	1.45
	II	73.8	1.06[1]	71.0	1.45
	III	73.5	0.81	72.2	1.76
	IV	73.8	0.56[1]	72.1	0.76
	Average	73.5	0.82	72.0	1.36
Ash in Flour Percentage (15% moisture basis)	I	0.398	0.014[1]	0.455	0.016
	II	0.406	0.008[1]	0.476	0.036
	III	0.399	0.014[1]	0.435	0.017
	IV	0.396	0.010[1]	0.453	0.017
	Average	0.400	0.012	0.455	0.022
Protein in Flour Percentage (15% moisture basis)	I	9.81	0.060[1]	10.05	0.081
	II	9.91	0.052[1]	10.13	0.098
	III	9.78	0.268	9.83	0.174
	IV	9.61	0.104	9.70	0.168
	Average	9.78	0.121	9.93	0.130
Diastatic Activity (Blish and Sandstedt method)	I	130.0	6.02[1]	165.0	4.56
	II	127.0	2.27[1]	171.0	12.25
	III	132.0	10.00[1]	166.0	8.52
	IV	123.0	5.49[1]	165.0	6.07
	Average	128.0	5.94	167.0	7.85

[1] The difference between the mills is significant.

performance when handled by trained operators. One would expect that the individuality of the millers would show up more with the less rigidly fixed flow of the Allis mill than with the inflexible flow of the Buhler.

Discussion

Assuming that the standard errors given in Table I are reasonably good estimates of the replication variability of the two mills, one may compute the magnitude of differences that would be necessary to

TABLE II

DIFFERENCES BETWEEN ANY TWO SINGLE DETERMINATIONS REQUIRED FOR SIGNIFICANCE

	Allis-Chalmers	Buhler
Flour yield, %	2.32	3.85
Ash, %	0.034	0.062
Protein, %	0.34	0.37
Diastatic activity	16.80	22.2

distinguish, definitely, two samples of wheat, or in other words, the significant difference. As it is not customary to replicate experimental millings, the significant differences have been calculated on the basis of single determinations, using the formula: significant difference between single determinations equals S.E. $\times 2\sqrt{2}$.

These values are large for both mills, particularly with respect to flour yield and diastatic activity. They indicate that the data on flours produced by single millings ought to be used with a great deal of circumspection.

It should be emphasized that the conditions under which this experiment was conducted were quite variable, especially in respect to temperature and relative humidity of the mill room. However, they were not unlike those occurring in many laboratories and the data herein presented should therefore be useful to the majority of those cereal chemists who have to do experimental milling. Shollenberger (1921), Geddes and West (1930), and others have shown that both temperature and relative humidity need to be accurately controlled if the replication error of the milling procedure is to be reduced to low values.

The estimates of error given in this paper probably represent maximum values to be anticipated with experienced millers working in rooms without control of temperature or humidity. Under the conditions indicated, the Buhler mill certainly exhibits a greater variability than the Allis. Its performance under accurately fixed conditions will be discussed in connection with the second experiment.

Offsetting the disadvantage of poorer replicability is the fact that the Buhler makes an acceptable experimental flour of good extraction, reasonably low ash, and higher diastatic activity than that from the Allis and, above all these considerations, is very much faster in operation.

Summary of the First Experiment

Seventy-two 2,000-gram samples were milled from one uniform lot of wheat, in a comparison of the replicability of the Allis-Chalmers and the Buhler experimental mills, by two operators, each having had wide experience with each mill.

The variability of the Buhler was higher in practically all cases than that of the Allis. There was very little evidence of differences in variability between millers. The Buhler mill is easier and faster to operate than the Allis.

Second Experiment, with Controlled Temperature

Shortly after the foregoing experiment was completed, air-conditioning equipment was installed in the mill room, and it was thought

advisable to conduct another test of the two mills under controlled temperature conditions. The experiment as performed differed somewhat from the plan of the former one. Two varieties of wheat were used, and three millers participated. One miller was highly experienced, one had had a small amount of experience with each mill, while the third had had no routine experience whatsoever.[5] Each operator alternated on the mills. As in the former experiment, different numbers of samples were milled on each mill because the Buhler mill is more rapid in operation than the Allis mill; ten samples per day were milled on the former and six on the latter. In each day's milling half the samples were of each of the two varieties. The general plan of the experiment can be seen from Table III in which is presented the average flour extraction, protein, and ash contents.

TABLE III

MEANS OF FLOUR EXTRACTION, PROTEIN, AND ASH CONTENT

Variety	Buhler mill—Miller				Allis mill—Miller			
	X	Y	Z	Av.	X	Y	Z	Av.
				Flour Extraction[1] %				
B	72.3	73.4	70.1	71.9	70.4	69.5	68.8	69.6
C	75.8	77.0	75.1	76.0	69.8	71.3	71.0	70.7
				Protein Content[1] %				
B	13.4	13.0	12.7	13.0	13.2	12.9	12.3	12.8
C	12.5	12.2	12.1	12.3	12.1	12.2	11.9	12.1
				Ash Content[1] %				
B	0.47	0.45	0.40	0.44	0.39	0.39	0.37	0.38
C	0.49	0.45	0.41	0.45	0.38	0.39	0.38	0.38

[1] Moisture basis 13½%.

Considering the averages given in Table III the most striking thing is the greater differentiation obtained between varieties B and C with the Buhler mill than with the Allis mill. With the Buhler, all three operators obtained significantly higher flour yields with variety C than with B; on the other hand there was no appreciable difference between these two wheats when milled on the Allis mill.

Table IV gives the standard errors of replication for the second experiment. As might be expected some of the errors are larger than in the previous experiment. On the other hand, it is noticeable that there is very little differentiation between the two mills, with both experienced and inexperienced operators. The only instance of significance was in the case of the flour protein.

As a final check on the results of the second experiment bakings were made of each variety milled by each miller on each mill the

[5] Each miller had a sound fundamental knowledge of the principles involved but his experience in using the mills with different wheat varieties was as stated above.

TABLE IV

STANDARD ERRORS OF REPLICATION OF FLOUR EXTRACTION, FLOUR PROTEIN, AND FLOUR ASH, WITH BOTH EXPERIENCED AND INEXPERIENCED MILLERS

	Buhler mill—Miller				Allis mill—Miller			
	X	Y	Z	Av.	X	Y	Z	Av.
Flour extraction, [1] %	1.5	0.8	0.8	1.0	1.3	1.6	0.7	1.2
Flour protein, [1] %	0.25	0.21	0.38	0.28	0.21	0.18	0.10 [2]	0.15
Flour ash, [1] %	0.019	0.008	0.014	0.013	0.013	0.006	0.013	0.011

[1] Moisture basis 13½%.
[2] The difference between mills is significant.

second day. Only the samples that represented the high and low extractions for the day were baked. The flours were baked unbleached but had been matured by storage at room temperature.

The following commercial-type straight-dough formula was used:

	Grams	Percentage based on flour
Flour	200 (15% m.b.)	100
Water	As required	...
Sugar	10	5.0
Salt	3	1.5
Yeast	4	2.0
Shortening	6	3.0
Dry skimmilk	8	4.0
Potassium bromate	0.002	0.001

The doughs were mixed to optimum development with the Swanson mixer, divided into duplicate loaves, fermented at 30° C. for 3 hours (105 minutes to the first punch, 50 minutes to the second punch and 25 minutes to the pan). They were proofed at 30° C. for 55 minutes and baked for 25 minutes at 420° F.

The baking data obtained are presented in Table V. There was no appreciable difference in baking quality of the flour from either mill or from any of the millers on any one mill, which may be due in part to the fact that both varieties had very poor baking qualities.

Summary of Second Experiment

Ten samples of each variety were milled by each of three millers (one experienced, one slightly experienced, and one inexperienced) on the Buhler mill and six samples of each variety on the Allis mill.

The flours were examined with respect to the percentage of flour extraction ascribable to millers and mills. On the Allis mill the experimental error more than covered differences of extractions between varieties. There was no significant difference due to either miller or mill as far as baking quality was concerned, which may be due in part to the extremely poor baking quality of both varieties.

TABLE V

BAKING DATA ON FLOURS FROM BUHLER AND ALLIS-CHALMERS MILLS [1]—2000-GRAM SAMPLES

Miller	Sample B				Sample C			
	Flour extraction	Water absorption	Grain and texture score	Loaf volume	Flour extraction	Water absorption	Grain and texture score	Loaf volume
	%	%		cc.	%	%		cc.
				Buhler Mill				
X	72.1	62	75F+	700	77.5	59	55VP	570
X	70.3	62	75F+	690	75.6	59	55VP	555
Y	73.2	63	70F+	675	76.8	58	55VP	535
Y	71.5	63	75F+	685	76.3	60	55VP	555
Z	70.2	62	75F+	690	73.4	57	55VP	560
Z	70.0	61	80F+	670	73.5	57	55VP	555
				Allis-Chalmers Mill				
X	72.6	62	75F+	700	68.4	57	55VP	545
X	68.8	63	80F+	695	70.1	58	55VP	540
Y	72.5	61	75F+	715	73.1	58	55VP	555
Y	70.8	60	80F+	710	71.6	60	55VP	565
Z	68.0	60	80F+	670	71.4	56	55VP	535
Z	69.0	61	80F+	680	70.8	57	55VP	550

[1] These data were secured from K. F. Finney, Baking Technologist, Hard Winter Wheat Quality Laboratory.

General Summary and Conclusions

The following general conclusions seem to be justified:

The variabilities of yield, ash, and diastatic activity are greater for the Buhler mill than for the Allis mill when the millers are skilled operators and the atmospheric conditions of the mill room are not controlled. These differences although not great are significant.

With the milling room air conditioned, and with both experienced and inexperienced millers, there were no significant differences between mills or millers, except with respect to flour protein, where one miller had better results on the Allis mill.

On the other hand, differences between wheats of different milling quality show up clearly with the Buhler mill when they may not be detectable by means of the Allis mill. Whatever the differences between the flours produced on the two mills, they were not great enough to be detected by the baking test.

The greater speed and ease of operation of the Buhler mill, together with its very compact construction, commend it to cereal technologists, especially where the volume of routine work is large.

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A COMPARISON BETWEEN THE ALLIS-CHALMERS AND MICRO-MILLING TECHNIQUES ON NORTH DAKOTA HARD RED SPRING WHEATS

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A description of a small or micro experimental flour mill suitable for the production of satisfactory flour from relatively small samples of wheat was reported by Geddes and Frisell (1935). This mill was designed to fulfill the need for a method of producing flour comparable in character to that yielded by the regular experimental mill but which would require a relatively small sample of wheat. By the use of this technique, in conjunction with a baking procedure which would yield miniature loaves and require a small quantity of flour, tests of milling and baking quality could be made available much earlier in the wheat-breeding program than had been possible with the older, standard experimental methods.

A study of the chemical attributes and baking quality of flours milled by the two procedures from the same series of 28 wheats of widely different protein content and baking strength was made by Geddes and Aitken (1935). The smaller mill was found to give a lower yield of flour and lower recovery of total products, but the flours were equal to those produced by the Allis mill in protein content. The micro-mill flours were higher in ash and carotene content, and slightly higher in diastatic activity. The milling losses were 3.4% and 1.0% respectively. Correlation coefficients for flour yield, protein,

ash, carotene, diastatic activity, and loaf volume for the flours produced by the two milling methods were very high and showed marked significant relationships for these factors in the two sets of flour. Difficulty was experienced, however, in scoring internal factors with the miniature loaves.

Harris and Sanderson (1938) investigated the merits of the miniature 25-gram baking procedure as compared with the 100-gram method. A series of 76 flours milled on the Allis-Chalmers experimental mill was employed as experimental material. Significant positive correlations were found between wheat protein and loaf volume for the two baking methods, and a relatively high positive correlation of +.8068 between the loaf volumes produced by the two methods. The authors concluded that this relationship would be useful in differentiating between strong and weak wheat varieties, but postulated that the surface of the cut loaf was too small to score satisfactorily for crumb color and texture.

VanScoyk (1937, 1939) found that the micro-baking technique gave as informative data as the larger-loaf procedure. Mechanical molding equipment was employed and no difficulty was experienced in obtaining satisfactory differentiation of both external and internal characteristics.

A special grant by the North Dakota Legislature in 1937 for the purchase of milling equipment made possible the purchase of a micro mill of similar construction to the one used by Geddes, Frisell, and Aitken. It was deemed advisable to investigate a series of flours milled from hard red spring wheats, grown in North Dakota, by the older Allis-Chalmers experimental mill, and to compare their chemical and baking characteristics with a similar series milled from the same wheats by the micro technique. In this way, knowledge regarding the relative performance of the two mills on North Dakota wheat would be gained, thereby indicating the suitability of the new micro technique in evaluating new wheats for the plant breeders.

Experimental Material and Methods

A group of 26 samples, each one of which contained a sufficient quantity of hard red spring wheat for millings on both the Allis-Chalmers and the micro mill, was selected for the purposes of this investigation. These wheats were free from damage of any kind, and graded relatively high, only one sample grading lower than No. 1 dark northern spring. The samples were cleaned, scoured, and tempered to a moisture content of 15% previous to milling. The milling techniques employed were very similar for both mills, with the exception that smaller quantities of wheat were milled in the instance of the

micro mill, and that only one corrugated roll was available, in this instance, as compared with the two sets of corrugated rolls in the Allis set-up.

The resultant flours from both mills were analyzed for moisture, protein, and ash, and for diastatic activity. The flours were baked by the micro procedure with the malt-phosphate-bromate formula, as this method has been shown to give maximum differentiation between samples. The moisture determinations were made in a Freas electric oven at 130° ± 3°C. for one hour. The ash was run by the magnesium acetate method in an electric muffle at 750°C. for one hour. The Blish-Sandstedt ferricyanide method as adapted by Sandstedt (1937) was used to determine the diastatic activity.

Discussion

The grades, test weight per bushel, wheat protein, flour yields, and total recovery of products are shown in Table I. The test weight of

TABLE I

GRADE, TEST WEIGHT, WHEAT PROTEIN AND MILLING DATA

Arranged in Order of Increasing Wheat Protein

Lab. No.	Grade [1]	Test weight	Wheat protein [2] (N×5.7)	Flour yield		Total recovery of products [2]	
				Allis	Micro	Allis	Micro
		Lbs. per bu.	%	%	%	%	%
11	2 DNS	57.4	13.3	72.1	73.0	99.0	99.3
14	1 HDNS	61.0	13.4	75.3	77.0	98.6	98.4
13	1 HDNS	60.6	13.5	75.8	77.8	99.0	99.6
9	1 HDNS	62.0	13.6	78.4	77.5	99.0	98.3
20	1 DNS	59.5	13.7	74.3	72.5	98.8	98.3
12	1 HDNS	60.7	13.8	75.4	76.9	99.1	99.5
22	1 HDNS	61.3	13.8	76.3	75.4	99.3	100.2
1	1 DNS	58.6	13.9	75.0	70.4	98.6	97.0
15	1 DNS	59.5	13.9	77.1	74.7	99.1	97.6
18	1 HDNS	60.8	14.1	77.2	77.8	99.1	98.3
4	1 DNS	59.6	14.2	75.7	74.1	99.1	97.4
5	1 HDNS	60.4	14.2	75.0	72.0	99.1	98.4
19	1 HDNS	60.3	14.2	75.6	75.1	99.6	97.8
16	1 DNS	59.3	14.3	76.7	77.2	99.7	99.7
21	1 HDNS	62.1	14.3	76.4	74.3	99.2	98.0
3	1 HDNS	60.6	14.4	76.1	74.0	98.6	100.2
7	1 HDNS	60.8	14.4	76.2	77.0	100.2	98.8
23	1 HDNS	61.5	14.4	76.0	75.8	99.8	97.4
10	1 HDNS	62.0	14.5	78.0	78.1	99.3	96.1
17	1 HDNS	61.2	14.5	77.9	77.4	99.8	99.1
25	1 HDNS	62.5	14.5	77.3	74.3	99.6	97.3
2	1 HDNS	62.0	14.6	75.9	72.5	99.1	97.5
8	1 HDNS	60.6	14.8	75.9	72.5	98.9	95.1
24	1 HDNS	62.0	14.8	76.1	76.3	98.8	98.9
6	1 HDNS	60.4	15.4	73.1	71.7	97.8	97.6
26	1 HDNS	60.0	17.0	74.6	75.0	99.1	98.2

[1] Unofficial.
[2] Moisture basis 13.5%.

these wheats ranged from 57.4 to 62.5, and the wheat protein from 13.3% to 17.0%. There was thus a small variation in test weight, but a more substantial difference in wheat protein. The flour yield varied from 72.1% to 78.4%, a range of 6.3% for the Allis-Chalmers experimental mill, and from 70.4% to 78.1%, a range of 7.7% in the case of the micro mill. In percentage of total recovery of products the values varied from 97.8% to 100.2% for the Allis, and from 95.1% to 100.2% for the micro mill. The corresponding ranges are 2.4% and 5.1% respectively. There accordingly appears to be greater variability in the yields obtained on the smaller unit, a conclusion borne out by the flour yield values, and the work of Geddes and Aitken (1935).

In Table II are shown the comparative data obtained from an analysis of the two series of flours. It is probable that the moisture percentages were not very pertinent, as the wheats were tempered in different lots for each mill, and accordingly these values are not given. The flour protein percentages appear quite similar, but differences are

TABLE II

COMPARATIVE DATA OBTAINED FROM THE TWO SERIES OF FLOURS
Arranged in Order of Increasing Wheat Protein

Lab. No.	Absorption	Loaf volume		Protein (N×5.7) [1]		Ash [1]		Diastatic activity [1]	
		Allis	Micro	Allis	Micro	Allis	Micro	Allis	Micro
	%	*cc.*	*cc.*	%	%	%	%	%	%
11	60	154	142	12.5	12.7	0.51	0.72	117.9	160.0
14	60	133	145	12.5	13.2	.50	.67	122.1	151.5
13	62	136	150	12.6	12.8	.54	.76	139.8	167.8
9	60	137	148	13.1	12.9	.54	.71	111.5	124.8
20	60	162	162	12.6	13.3	.45	.67	97.2	115.4
12	60	148	146	12.7	13.3	.54	.74	90.7	111.2
22	61	142	136	13.0	13.6	.65	.63	143.3	185.6
1	62	136	151	13.4	13.2	.47	.65	103.9	106.6
15	57	128	133	12.5	13.2	.50	.67	91.2	124.5
18	62	132	140	13.1	13.3	.54	.72	134.0	179.6
4	65	132	139	13.4	13.4	.53	.81	128.1	161.4
5	64	122	139	13.5	13.7	.61	.80	145.0	172.8
19	60	117	125	13.2	13.4	.52	.72	146.7	177.2
16	60	145	138	13.0	13.2	.53	.65	88.6	114.3
21	60	162	145	13.1	13.4	.50	.73	97.0	125.6
3	61	127	142	13.6	13.3	.52	.66	100.3	117.4
7	60	144	156	13.5	13.9	.39	.72	87.5	110.2
23	60	148	130	13.3	13.9	.51	.83	119.7	167.7
10	62	137	137	12.6	14.0	.58	.84	136.2	194.3
17	60	148	159	14.0	13.8	.52	.75	115.1	153.4
25	60	148	148	13.5	13.6	.68	.68	102.3	132.6
2	61	127	172	13.4	13.8	.54	.73	94.0	123.2
8	60	145	145	13.5	13.7	.37	.68	88.4	120.2
24	60	165	138	13.5	14.0	.41	.65	107.4	133.4
6	59	111	118	14.5	14.6	.41	.69	79.0	99.4
26	60	170	154	16.2	16.2	.42	.84	99.5	140.3

[1] Moisture basis 13.5%.

apparent in ash and diastatic activity between the flours produced by the two milling procedures.

Table III shows the means, differences between means, standard deviations, and coefficients of variability computed from the data. The same absorption was shown for each pair of flours regardless of the milling method. With the exception of protein and loaf volume, the micro-mill results appear to be more variable than the Allis values. The differences between mean flour protein, flour yield, and loaf volume are clearly not significant. Flour ash and diastatic activity are

TABLE III

TABLE OF STATISTICAL CONSTANTS

Means, Standard Deviations and Coefficients of Variability

	Means			Standard deviations		Coefficients of variability	
	Allis	Micro	Difference [1]	Allis	Micro	Allis	Micro
Flour protein %	13.30	13.58	0.28	0.752	0.676	5.65	4.98
Flour yield %	75.90	75.01	0.89	1.385	2.174	1.82	2.90
Total recovery %	99.13	98.23	**0.90**	0.473	1.174	4.77	11.95
Flour ash %	0.51	0.72	**0.21**	0.073	0.195	14.31	27.08
Diastatic activity	111.02	141.17	**30.15**	20.07	27.343	18.08	19.37
Loaf volume *cc.*	140.62	143.77	3.15	14.422	11.288	10.26	7.85
Moisture %	14.35	13.96	**0.38**	0.282	0.299	1.96	2.14

[1] Significant differences are shown in heavier type.

significantly higher for the micro flours. The same tendency was noted by Geddes and Aitken (1935), although their mean differences were not found to be significant in the case of diastatic activity. In the present investigation, it is extremely probable that sharper rolls on the new micro mill as compared with the duller rolls on the Allis, which had been in use over a comparatively long period, materially increased the spread between these means. Moisture content of flour and total recovery of products were higher for the Allis procedure. Geddes and Aitken found that the micro method had a higher loss, and therefore lower total recovery, than the Allis procedure.

The correlation coefficients calculated from the data are shown in Table IV. Significant positive correlations between flour protein, flour yield, diastatic activity, and loaf volume are demonstrated, although the relationship between the two series of loaf volumes is lower than one would expect from a knowledge of the results obtained in a similar investigation conducted by Geddes and Aitken (1935), and of the conclusions reached by Harris and Sanderson (1938). It must be borne in mind, however, that Geddes and Aitken worked with wheats of widely different protein content and baking strengths, whereas the

TABLE IV

CORRELATION COEFFICIENTS COMPUTED FROM THE COMPARATIVE DATA

Variables correlated		Correlation coefficient	
X	*Y*	r_{xy}	*P* [1]
Flour protein %	Flour protein %	**+.8815** [2]	<.0001
Flour yield %	Flour yield %	**+.6075**	.0010
Total recovery %	Total recovery %	+.0914	>.5542
Flour ash %	Flour ash %	+.0325	>.5542
Diastatic activity	Diastatic activity	**+.9263**	<.0001
Loaf volume (Allis) *cc.*	Loaf volume (micro) *cc.*	**+.4025**	.0420
Test weight	Flour yield (Allis-Chalmers) %	**+.6811**	.0002
Test weight	Flour yield (micro) %	**+.4221**	.0318

[1] Probability of the observed correlation coefficient arising from uncorrelated material through errors of random sampling.
[2] Significant correlation coefficients are shown in heavier type.

wheats employed in the present investigation all belonged to the hard red spring class, and did not vary widely in strength. Harris and Sanderson used only a series of flours milled on the Allis-Chalmers, so the results reported by these two pairs of independent investigators are not strictly comparable to the data contained in this study. A summary of the color and texture scores revealed little difference between the two methods in respect to these characteristics.

Summary and Conclusions

A series of 26 samples of hard red spring wheat were milled into straight flour by two experimental procedures. One was the usual method, using 2,000 grams of wheat which was milled in an Allis-Chalmers mill consisting of two corrugated rolls and one smooth roll, while the other was the micro-milling procedure described by Geddes and Frisell (1935). The resultant flours were analyzed for protein, ash, moisture, and diastatic activity, and were baked into miniature loaves employing doughs made from 25 grams of flour.

An analysis of the data showed no significant differences between the mean values of the two series of flours in respect to protein content, flour yield, and loaf volume. Significant differences in means, however, were shown for flour ash and diastatic activity, the micro-milled flour being highest in each instance. It is extremely probable that the sharper rolls on the micro mill, as compared with the Allis, affected these flour characteristics.

Significant positive correlation between milling methods was demonstrated for flour protein, flour yield, diastatic activity, and loaf volume, although the majority of the coefficients were not sufficiently high to justify predicting one variable from a knowledge of the other. It is probable that a wider range in flour strength would increase the correlation between loaf volumes.

Acknowledgments

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VARIATIONS IN DOUGH-DEVELOPMENT CURVES

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The use of physical methods, particularly recording dough mixers, in the testing of wheat and flour is restricted in comparison with the use of chemical determinations and the baking tests. While the users of chemical and baking tests are numbered in the hundreds, the number of users of recording dough mixers is limited, at least in North America, to a few dozen. Greater progress has been made in perfecting the mechanical devices for making these physical tests than in the interpretation of their results in terms of baking value. One of the reasons for the slowness in learning the interpretation of these dough-development curves is the comparatively small number who use the curves and the consequent limited exchange of ideas concerning their meaning in practical use. Another difficulty is the lack of an adequate standard for interpreting these curves.

Studies in Dough Development

The watt meter was used by Swanson and Working (1933) in recording the behavior of dough during development. St. John and Bailey (1929) attached a watt meter to a dough-mixing machine and by this means measured the power requirements for mixing. These authors used Bingham and Murray's (1923) method in measuring the

[1] Contribution No. 59, Department of Milling Industry.

plasticity. The relationship of mixing speed to dough development was studied by Stamberg and Bailey (1938), who state that a medium to moderately high speed resulted in superior bread. Halton and Scott Blair (1937) studied physical properties of flour in relation to their bread-making qualities and they state that the two properties which are of chief importance are viscosity and elasticity modulus. Malloch (1938) devised a recording mixer which operates on flour samples containing only 7 grams of dry matter. The curves obtained showed a break in curvature which varied in position and sharpness for the different flours. There were indications that the time of the break is related to gluten quality. Bohn and Bailey (1936) used a "stress meter" to study the stress-strain relation of flour dough, "including a study of plastic flow by measuring the dying out of stress with time of stretching the dough." It was found that strong flours gave higher stress readings than weak flours and that high stress-meter readings are a good indication of the ability of dough to withstand prolonged mixing. Schofield and Scott Blair (1932, 1933, and 1933) studied the relationship between viscosity, elasticity, and plastic strength of dough, using an apparatus by which a piece of dough could be stretched for varying lengths of time, and also noted the dying-out stress.

Hogarth's Device

The knowledge of a mechanism for testing and recording the physical properties of flour existed about the time cereal laboratories were first started in connection with flour mills in the United States. An application for a patent on such a device was filed by J. Hogarth in the United States patent office in 1890 and the patent was granted May 10, 1892, letters patent No. 474,636. It is stated that this invention has appliances for mechanically testing and sampling different qualities of flour and graphically indicating and recording the various characteristics or properties of the different flours tested. The dough was mixed in a kneader of the Werner-Pfleiderer type and the resistance of the dough was recorded on cross-section paper using the dynamometer principle. There was also provision for determining the amount of water required to produce a dough of desired consistency. Cereal chemists are still looking for a satisfactory method of measuring water absorption.

Extensimeters

Hogarth's device apparently did not become well known since it does not seem to be mentioned in the literature of the cereal chemists.[2] The extensimeter designed by Chopin (Bailey and Le Vesconte, 1924;

[2] The author is indebted to Dr. C. H. Bailey for calling attention to Hogarth's invention

Chopin, 1927) measures the tensile strength of a sheet of dough when blown into a bubble by means of expanding air under accurately controlled pressure. The Comparator, made by Buhler Brothers, Switzerland, is also a device to measure the tensile strength of a sheet of dough when subjected to the pressure exerted by a rounded, truncated cone. Kress (1924) reports a device by James to record in a curve the resistance of wet gluten to stretching, the distance it can be stretched before breaking, and the character of the break whether gradual or sharp. Hankóczy (1920) used this principle on the wet crude gluten. The ideas underlying these devices were that the volume and behavior of the bubble were related to the baking strength of the flour. Other references to physical methods are given by Swanson (1938).

Recording Dough Mixer

The farinograph (Brabender 1932, 1934) embodies several ideas found in Hogarth's device. Hankóczy developed a machine for determining the physical properties of dough in order to have a laboratory method which would give information on the qualities of the Hungarian wheat crop. He needed especially a device to determine absorption.[3] The Swanson-Working dough mixer (Swanson and Working, 1933) was designed to measure and record automatically the rate of dough development, the duration of resistance against mechanical action, and the rate and extent of increase in mobility of dough as a result of mechanical action. That these characteristics are related to inheritance was shown by Swanson (1936) and in so far as this is true, the dough-mixer curves serve one of the primary needs of wheat improvement work, namely, to differentiate characteristics due to inheritance. The distinctive and, as far as known, original feature of the recording dough mixer is the principle of mixing. As soon as the water films on the flour particles have become uniformly distributed in the dough so as to develop the elastic, plastic, and viscous properties, the dough is subjected to a continuous stretching, folding, and restretching action. This subjects a small body of dough to a treatment which is similar to that given in large mixers where the force of gravity serves the same function as the fixed pins in the bowl of the recording mixer, namely, to hold the dough back while being stretched and folded by the upper pins which have a planetary motion.

Mobility in Dough Structure

The characteristics of the curves depend on the condition of the mobility of dough constituents in relation to each other and the

[3] Private communication.

changes in this mobility during the progress of mixing. This mobility is influenced by the rate of wetting or hydration of the flour particles, the development and orientation of the gluten strands, and the conditions which influence plasticity or the movement or sliding of particles relative to each other. There is usually at first a gradually increasing resistance to the movement of the revolving pins and the amount of this resistance is related to the height of the curve. Continued mixing beyond the point of maximum resistance generally causes an increase in the mobility. This may be due either to the breaking of the gluten strands or to the disintegration of the colloidal complex. Markley and Bailey (1938) conclude that "the use of this rate of increase in mobility upon prolonged mixing is impractical as a simple and direct measure of flour strength, since it is the resultant of so many variable factors."

Curve Characteristics

Experience with pure varieties of different types has shown that the curve characteristics most closely related to baking phenomena are steepness of the ascending slope, the time required to reach the peak, the character of this peak whether sharp or rounded, and the general width and height of the curve. The steepness of the descending slope and the narrowing of the curve due to increased mobility, which to a certain extent indicates the rate and extent of the breakdown of the dough structure, have also a value. Since mixing for baking usually is not carried to the point of increased mobility in dough, this can have only a theoretical value as an indication of inherent dough structure. The time between any important points in the curves presented in the accompanying illustrations can be estimated. The distance between the curved lines of the graphs represents three minutes of elapsed time.

It should be clearly understood that curve characteristics from different recording mixers will not be similar unless the adjustments and conditions of operation are the same. How much curve characteristics may differ simply as a result of adjustments can be seen by comparing the curves shown by Swanson (1936) with those shown by Swanson and Clark (1936) as well as by Swanson (1938). Absorption or the amount of water used in mixing the dough has also important effects. Thus curves made in different laboratories should not be compared at least in minor details unless it is known that the adjustments and conditions of operation are also comparable. This situation adds another problem in cooperative work with dough mixer curves.

Variations in Curves

In the previous papers (Swanson and Working, 1933; Swanson, 1936) the possibilities of using the curves as indicating characteristics due to heredity were stressed. In the continued use of the curves in connection with wheat-variety studies it has become evident that while the curves of various varieties follow a certain pattern, there may be very wide variations which must be recognized. Thus it has been found that different varieties may produce curves which are as much alike as if they were from the same variety, and curves from the same variety may vary as much as curves from different varieties. This is shown in the figures presented.

Causes of the Variation

The variations from a general type or pattern are due to weather conditions, particularly during heading and ripening, and the protein content. The protein content has a marked influence on the height and general shape of the curve. The main point in this paper is to show how much and in what respects dough curves may be influenced by environmental factors when wheats are grown in various places and in different years. The curves selected for this study were from three crop years, 1935, 1936, and 1937. These years represented wide variations in weather conditions, both annually and locally. The year 1936 was extremely hot and dry in Kansas. The other two years were more nearly normal.

It should be clearly understood that these curves were not made as part of a planned experiment but were selected from tests made on varieties grown in the three years. All of the curves selected for illustration and study were from wheats grown in Kansas except the four spring varieties which were included for comparison.

General Plan for Presentation of Curves

The general plan followed in selecting the curves presented in this paper was first to find one or more curves from each of the varieties mentioned below which had the general pattern characteristic of most curves obtained on that variety. This pattern is based on the mental picture obtained from the study of a large number of curves from the various varieties, and it means that most of the wheats of average protein content and grown under normal weather conditions will give curves which are similar for a particular variety. Next, curves were selected which showed the greatest deviation from this general pattern. Besides these, curves were chosen to represent various intermediate types. These groups of curves were then mounted and photographed.

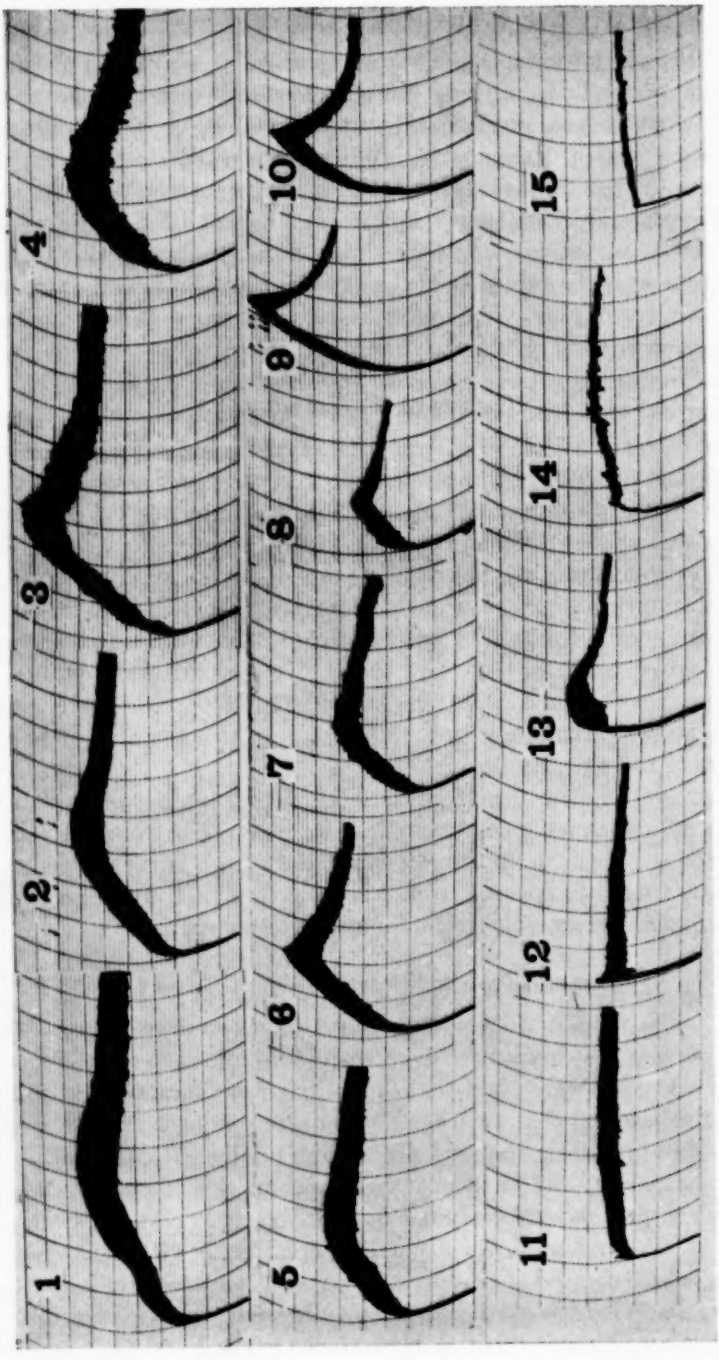

Fig. 1. Group of curves showing a wide range in characteristics.

The curves from the different varieties which showed the widest differences were chosen.

The following varieties are represented in Figures 2 to 9.

Hard Red Winter Wheats		*Hard Red Spring Wheats*	*Soft Red Winter Wheats*
Turkey	Blackhull	Marquis	Fulcaster
Kanred	Chiefkan	Ceres	Kawvale (semi hard)
Tenmarq	Early Blackhull	Reward	Harvest Queen
Cheyenne		Thatcher	Clarkan

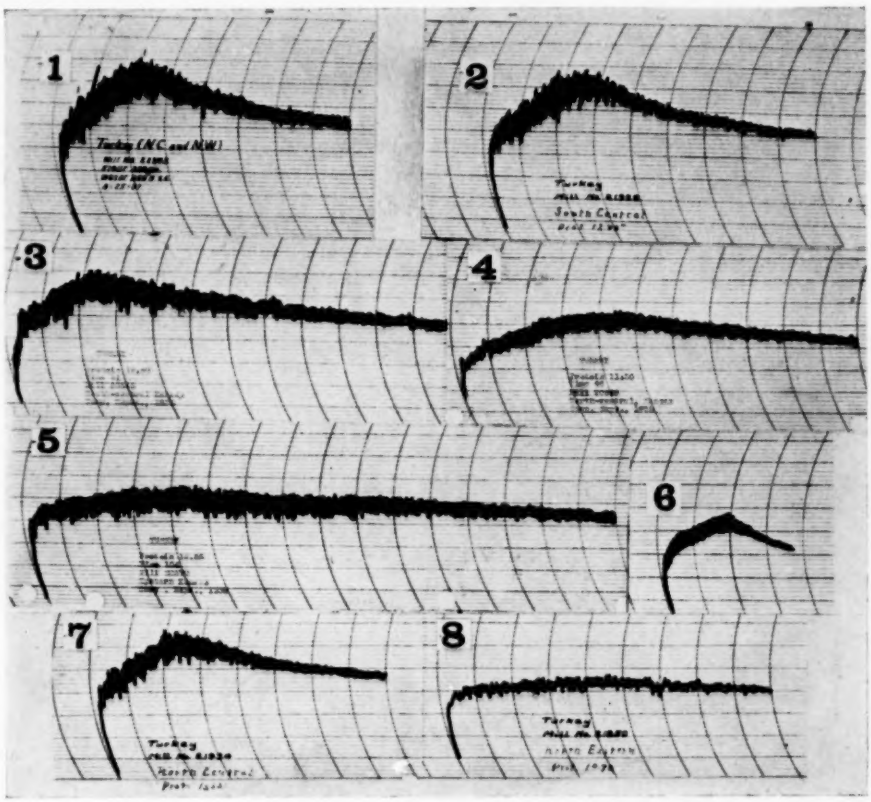

Fig. 2. Turkey flours.

No.	Sample	Place grown in Kansas	Year	Test weight
				Lbs.
1	22903	N. Central and N.W.	1937	59.2
2	21926	S. Central	1936	58.9
3	20555	S. Central	1935	56.3
4	20563	N. Central	1935	55.9
5	20572	Eastern	1935	56.8
6	22008	Hays Exp. Sta. (N. Central)	1936	59.2
7	21934	N. Central	1936	57.0
8	21950	N. East	1936	59.5

Main Curve Characteristics as Illustrated in Figure 1

In Figure 1 are presented various types of curves without any reference to variety. These 15 curves show the range of variations which are shown in the other figures presenting the curves of the various varieties. The four main characteristics in the curves are:

1. The steepness and length of the ascending slope, which indicates the rate and manner of dough development.

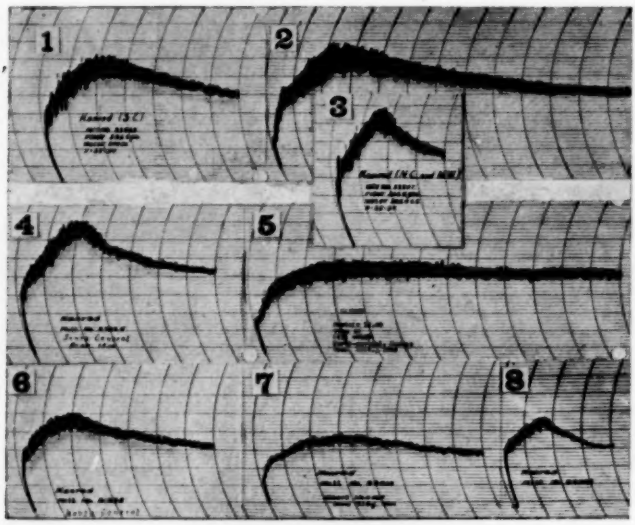

Fig. 3. Kanred flours.

No.	Sample	Place grown in Kansas	Year	Test weight
				Lbs.
1	22893	S. Central	1937	60.6
2	20554	S. Central	1935	54.6
3	22902	N. Central and N.W.	1937	58.7
4	21925	S. Central	1936	58.3
5	20562	N. Central	1935	54.8
6	21933	N. Central	1936	60.9
7	22010	Northwest	1936	52.5
8	22006	Hays Exp. Sta. (N. Central)	1936	58.6

2. The general shape of the turn at the top, which shows the duration of time during which the dough maintains its resistance against the mechanical force.

3. The general width of the curve, which is an indication of the elastic properties.

4. The steepness of the descending slope and the amount of narrowing of the curve, which indicate the rate and the extent of increase in the mobility.

Rate of Dough Development

The rate of dough development may be characterized as very slow in curves 1 and 2; slow in curves 3, 4, and 5; medium in curves 6, 7, and 8; rapid in curves 9 and 10; and very rapid in curves 12 and 13. The rate of dough development seems to be associated with the rate of wetting or hydration. Curves like 9 and 10 show a rapidly increasing resistance as the dough develops, while in curves like 11, 12, and 13 there is little or no increase in resistance after the dough begins to form. Doughs like 1 and 2 behave as though the gluten strands were longer and hence become arranged in a parallel pattern more slowly. The increase in resistance apparently takes place as

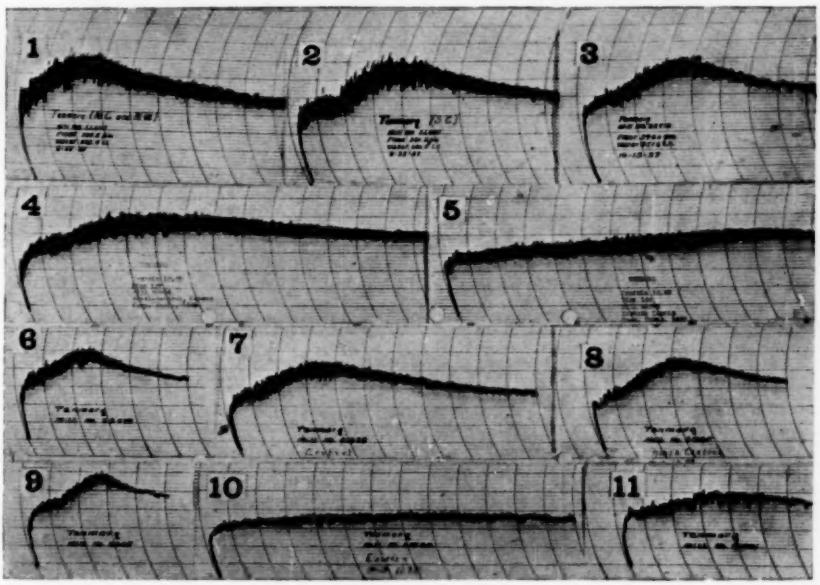

Fig. 4. Tenmarq flours.

No.	Sample	Place grown in Kansas	Year	Test weight
				Lbs.
1	22904	N. Central and N.W.	1937	58.8
2	22895	S. Central	1937	
3	22910	Central	1937	59.4
4	20564	N. Central	1935	56.9
5	20573	Eastern	1935	54.5
6	22009	Hays Exp. Sta. (N. Central)	1936	58.4
7	21938	Central	1936	57.5
8	21935	N. Central	1936	56.5
9	22005	Hays Exp. Sta. (N. Central)	1936	60.3
10	21944	Eastern	1936	57.9
11	21991	Manhattan (E. Central)	1936	58.8

more and more of these strands are oriented. At the point of maximum orientation in curves like 9 and 10, the dough begins to break rapidly. In curve 12, the wetting is almost immediate and in 11 and 13 very rapid, which means that the elastic properties of the dough are developed almost at once. Low and thin curves like 14 and 15 show a lack of dough development and the behavior is similar to that of soft putty. The plastic properties are so prominent as to submerge the elastic. Curves like 14 and 15 indicate a pronounced abnormal

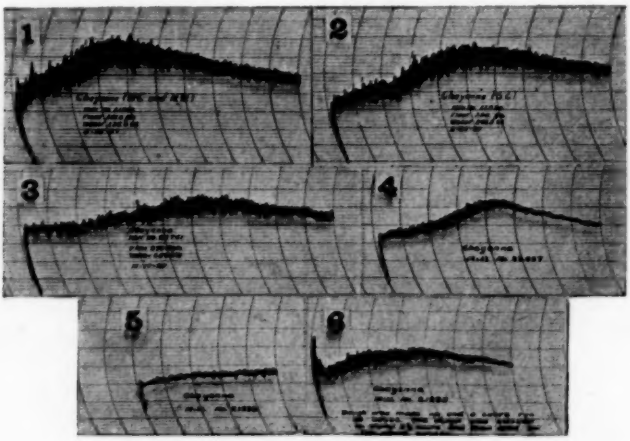

Fig. 5. Cheyenne flours.

No.	Sample	Place grown in Kansas	Year	Test weight
				Lbs.
1	22905	N. Central and N.W.	1937	59.5
2	22896	S. Central	1937	60.5
3	22741	Manhattan (East Central)	1937	60.8
4	22007	Hays Exp. Sta. (N. Central)	1936	60.4
5	21993	Manhattan (East Central)	1936	59.6
6	21993	(Made after 4 hours in dough)	1936	59.6

condition, while curves like 11, 12, and 13 may be obtained from low-protein flours, 11 is from a low-protein hard wheat, and 12 and 13 are from soft wheats.

Top Characteristics

A very rounded top is represented by curves 1, 2, and 5, rounded by 3, 4, and 7, while 9 and 10 have a sharp top. A sharp top is generally accompanied by a rapid dough development showing that the stiffness increases very rapidly, but the duration of maximum

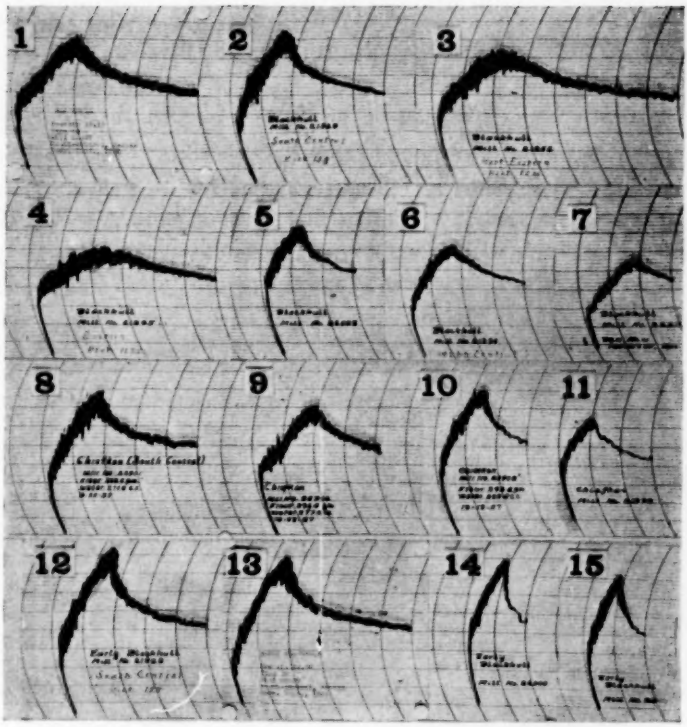

Fig. 6. Blackhull, Early Blackhull, and Chiefkan flours.

No.	Sample	Place grown in Kansas	Year	Test weight
				Lbs.
		Blackhull		
1	20557	S. Central	1935	59.2
2	21928	S. Central	1936	60.7
3	21952	Northeast	1936	61.2
4	21945	Eastern	1936	60.6
5	22003	Hays Exp. Sta. (N. Central)	1936	60.8
6	21936	N. Central	1936	58.6
7	22011	Northwest	1936	61.9
		Chiefkan		
8	22901	S. Central	1937	63.1
9	22744	Agronomy Farm, Manhattan (E. Central)	1937	63.6
10	22908	N. Central and N.W.	1937	61.7
11	21974	Agronomy Farm, Manhattan (E. Central)	1936	62.3
		Early Blackhull		
12	21929	S. Central	1936	61.8
13	20559	S. Central	1935	60.7
14	22000	Hays Exp. Sta. (N. Central)	1936	63.8
15	21960	Agronomy Farm, Manhattan (E. Central)	1936	63.2

resistance is very short because the increase in mobility is rapid. Curves 11, 12, 14, and 15 have no pronounced turn at the top.

Width of Curve

Curves 1, 3, and 4 may be characterized as wide or bold. Medium width is found in 2, 5, 6, 7, 11, and 12; narrow in 8, 9, and 10, and very narrow in 14 and 15. Width of curve or boldness is generally obtained from flours which are characterized as very strong or those having a wide tolerance to varying conditions of bread baking. Nar-

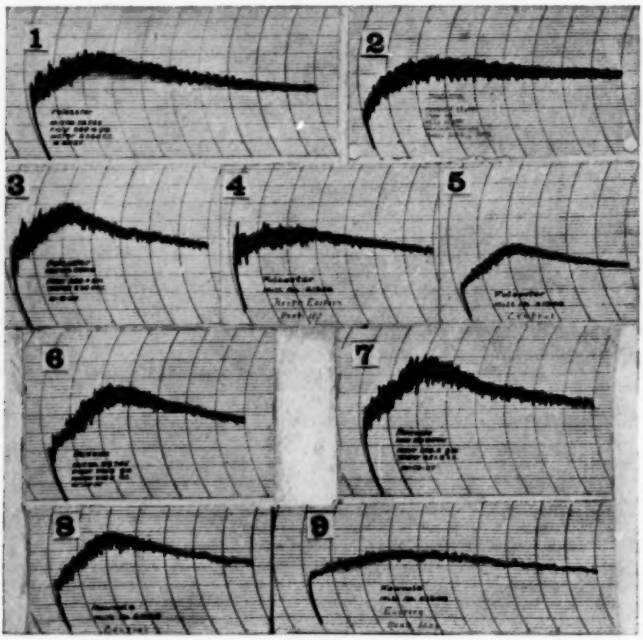

Fig. 7. Fulcaster and Kawvale flours.

No.	Sample	Place grown in Kansas	Year	Test weight
				Lbs.
		Fulcaster		
1	22746	Manhattan (East Central)	1937	61.4
2	20577	Eastern	1935	56.6
3	22912	Central	1937	53.7
4	21954	Northeast	1936	60.4
5	21940	Central	1936	57.7
		Kawvale		
6	22745	Manhattan (East Central)	1937	60.3
7	22911	Central	1937	59.9
8	21939	Central	1936	57.4
9	21946	Eastern	1936	58.5

row curves result especially after the peak has been passed, apparently because of a gluten structure which disintegrates more easily under mechanical action. The very narrow curves such as 14 and 15 are exceptional and indicate an abnormal condition.

Rate of Increase in Mobility

Curves 1, 3, 4, and 11 show very little increase in mobility. The gluten strands apparently continue to slide on each other without breaking. Curves 2, 7, and 12 show a medium rate of increase, while 6 and 8 show a rapid increase and in 9, 10, and 13 the increase in

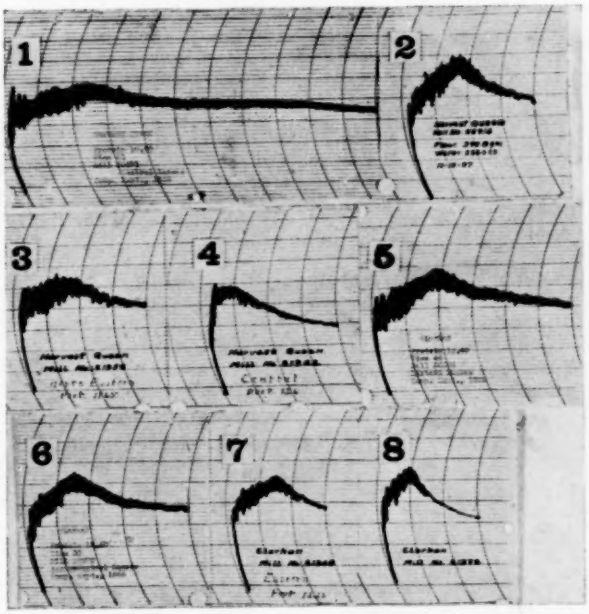

Fig. 8. Harvest Queen and Clarkan flours.

No.	Sample	Place grown in Kansas	Year	Test weight
				Lbs.
		Harvest Queen		
1	20571	Central	1935	55
2	22914	Central	1937	57.5
3	21956	Northeast	1936	59.3
4	21942	Central	1936	57.0
		Clarkan		
5	20578	Eastern	1935	59.0
6	20570	Central	1935	58.3
7	21948	Eastern	1936	60.1
8	21972	Manhattan (E. Central)	1936	61.3

mobility is very rapid. This rapid increase in mobility apparently is due either to a breaking of the gluten strands or to more pronounced thixotropic properties. Curves like 14 and 15 have a very small mobility and hence very little or no increase is evident.

Groups of Curves (Figs. 2 to 9)

The groupings in Figures 2 to 9 were made to bring out how much curves from the same variety may vary not only because of conditions of growth during heading and maturing but also on account of the soil, which is the most potent factor in the protein content. For this reason curves of the same variety were grouped on the same photo-

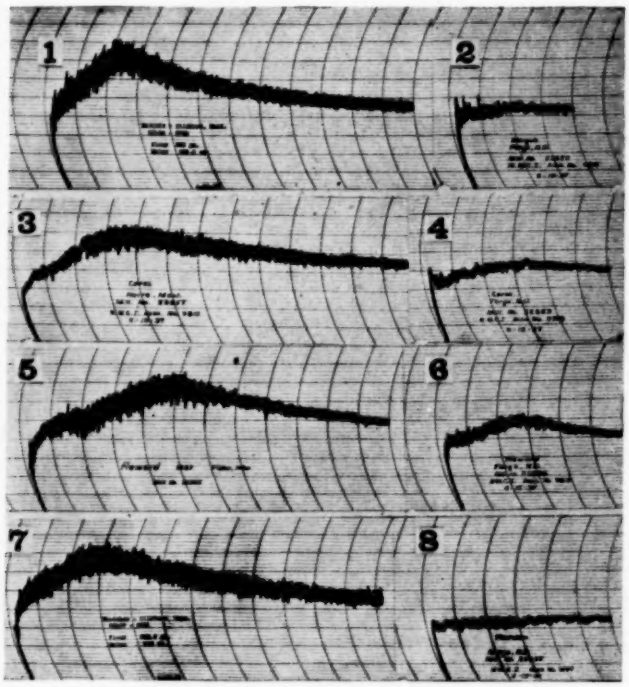

Fig. 9. Spring-wheat flours.

No.	Sample	Variety	Place grown	Year
1	23184	Marquis	Gildford, Mont.	1937
2	22652	Marquis	Fargo, N. D.	1936
3	22657	Ceres	Havre, Mont.	1936
4	22653	Ceres	Fargo, N. D.	1936
5	21345	Reward	Fisher, Minn.	1935
6	22654	Reward	Fargo, N. D.	1936
7	23187	Thatcher	Gildford, Mont.	1937
8	22655	Thatcher	Fargo, N. D.	1936

graph. An attempt is made in the following to place in groups those curves from different varieties which are similar.

The curves in the various figures may be placed in the following groups:

1. Strong bold curves similar to curves 1 to 5 in Figure 1.
2. Moderate short curves similar to curves 6 to 8 in Figure 1.
3. Short and sharp curves similar to curves 9 and 10 in Figure 1.
4. Very rounded or flat curves similar to 11 and 12, Figure 1.
5. Short or rapidly thinning curves similar to 13, Figure 1.
6. Low and thin curves similar to 14 and 15, Figure 1.

GROUP 1, STRONG AND BOLD

Fig. No.	*Variety*	*No. of curve*
2	Turkey	1, 2, and 3
3	Kanred	1 and 2
4	Tenmarq	1, 2, and 3
5	Cheyenne	1 and 2
7	Fulcaster	1, 2, and 3
	Kawvale	6 and 7
9	Marquis	1
	Ceres	3
	Reward	5
	Thatcher	7

GROUP 2, MODERATELY SHORT

Fig. No.	*Variety*	*No. of curve*
2	Turkey	6 and 7
3	Kanred	3, 4, and 6
4	Tenmarq	6
6	Blackhull	1, 3, and 4
7	Kawvale	8
8	Clarkan	5 and 6

GROUP 3, SHORT AND SHARP

Fig. No.	*Variety*	*No. of curve*
6	Blackhull	2 and 5
	Chiefkan	8 and 9
	Early Blackhull	12 and 13
8	Harvest Queen	2

GROUP 4, VERY ROUNDED OR FLAT

Fig. No.	*Variety*	*No. of curve*
2	Turkey	4 and 5
3	Kanred	5
4	Tenmarq	4, 5, and 7
5	Cheyenne	3
7	Fulcaster	4
8	Harvest Queen	1
9	Marquis	2
	Reward	6

GROUP 5, SHORT OR RAPIDLY THINNING

Fig. No.	*Variety*	*No. of curve*
3	Kanred	8
4	Tenmarq	8 and 9
5	Cheyenne	4
6	Blackhull	6 and 7
	Chiefkan	10 and 11
	Early Blackhull	14 and 15
8	Harvest Queen	3 and 4
	Clarkan	7 and 8

GROUP 6, LOW AND THIN

Fig. No.	*Variety*	*No. of curve*
2	Turkey	8
3	Kanred	7
4	Tenmarq	10 and 11
5	Cheyenne	5
7	Fulcaster	5
	Kawvale	9
9	Ceres	4
	Thatcher	8

Not all curves will fit into this classification since some are on the border line between groups, but this grouping shows that flours of various varieties may be similar in their behavior in mixing and likewise that flours of the same variety may be dissimilar. Blackhull, Chiefkan, and Early Blackhull as a group differ markedly from the other varieties although some of their curves find similarities in curves of other varieties.

The outstanding characteristics of the curves from these three wheats are the abrupt turn at the top and the rapid descent, accompanied by rapid narrowing. The two soft wheats, Harvest Queen

and Clarkan, also have as distinguishing characteristics the wideness of the curve at the start and the rapid narrowing during descent.

Discussion

In considering the usefulness of the curves, it should be emphasized again that these curves were selected to show the widest variability in order to bring out clearly how much curves from the same varieties may differ. These differences are caused by the growth conditions during heading and ripening, and by the protein content. Thus, curves of different varieties should not be judged unless the wheats have been grown under a similar environment and the protein contents are in the same range.

While protein content may influence curve characteristics and must be considered in judging curves, it is not necessarily a determining factor. As a rule low-protein flours will give curves which are flattened, or with tops very rounded and the height much decreased, while high-protein flours tend toward the opposite characteristics.

Critics may say that the curves made on the recording dough mixer have nothing in common with commercial practices, since the mixing goes much beyond the point reached in the commercial bakeshop. The reply to this statement is that the purpose of making these curves is not to imitate commercial practices but to discover physical characteristics of the varieties. In finding the breaking strength of materials it is necessary to go much beyond what it is expected these will have to withstand in practical operation. If the magnitude of the strain is such as to break only the weak, then it will not be known how much of a load the strong can carry. It is very evident that many wheats will yield flours which are stronger than needed in average bread baking. The quality tests should show just how strong these flours are so as to indicate how much strength they may give to the mix which contains the weaker flours. Dough-mixer curves may be used as one of such tests.

Another criticism which may be given on the curves presented is that since the curves can vary so much even in the same variety they have little value in variety testing. It has already been stated that those curves which showed the greatest variability were chosen for these figures. Experience over several years with curves made on varieties has shown that as a rule, probably with 90 per cent of the flours, the curves from any one variety grown under approximately similar conditions will present a pattern of characteristics which is associated with the particular variety tested. Certain varieties like Turkey and Kanred will show strong similarities; likewise will Cheyenne and Tenmarq. These last two will be more like the spring

wheats. Fulcaster and Kawvale, when grown under conditions that favor hard wheat, will give curves which are very similar to those of Turkey. Blackhull, Early Blackhull, and Chiefkan will produce curves which have strong similarities, those of Blackhull having as a rule less sharp tops than the other two. The soft wheats like Harvest Queen and Clarkan in most cases will show quick development of the dough and a rather rapid narrowing of the curve after the peak has been reached.

The use of physical tests is relatively new although, as has been pointed out, the principles have been known to a few people since the beginning of wheat and flour testing. The newness of the use of physical tests should not be held against them nor should other tests be favored simply because they have been used longer and more widely. These physical tests measure certain characteristics, and if they can show wider variation among flours than almost any other test, then the challenge is to learn their interpretation. Much cooperative work has been done to standardize and evaluate the various chemical and baking tests used in cereal laboratories, but little has been done with reference to the physical tests. Only a few cereal chemists have used them and very little collaborative work has been undertaken.

The most serious aspect is that we have thus far no adequate method of evaluating these physical tests. It is a question whether we shall learn the interpretation of these physical tests as long as we use, as a standard, baking-test methods based on formulas and procedures comparable to commercial practices. Such baking tests seem more or less to coddle the weak flours and do not give the strong ones a chance to show all of their possibilities. It may be that what we need is to get away from the idea of a commercially acceptable volume and texture and use a baking procedure the main purpose of which shall be to test the inherent possibilities the same as when engineers measure strength of materials.

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THE VITAMIN B_1 CONTENT OF WHEAT, FLOUR AND BREAD

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(Read at the Annual Meeting, May 1939)

It has long been known that the production of fine white flour involves the removal of certain otherwise desirable food factors. Prominent among these factors is vitamin B_1 or thiamin, as it is now known. The purpose of the present communication is to present the results of thiamin assays made on several mill streams. This study shows the distribution of thiamin in the various parts of the wheat kernel and also shows what may be expected in flours of various degrees of extraction. Thiamin was determined by our fermentation

method, which has been described elsewhere (Schultz, Atkin, and Frey, 1937, 1938). Substances like wheat, flour, and bread may be analyzed by our method with great ease.

The test takes only three hours and requires but little attention during that time. A number of tests can be run simultaneously. The thiamin does not have to be separated or isolated from the bulk of the sample, and furthermore the test is very sensitive, requiring only 2 to 4 micrograms of thiamin for a satisfactory test. The test is, however, not entirely specific since we have found that 2-methyl-5-ethoxymethyl-6-amino-pyrimidine also gives the test (Schultz, Atkin, and Frey, 1937). This pyrimidine represents a synthetic half of the thiamin molecule and has not yet been found in natural food products. If a product like whole wheat, for instance, is to be studied it is only necessary to have one or two samples analyzed by a reliable animal method. If the assay agrees with the fermentation method, then it may be assumed that interfering substances are absent. This is true in the case of wheat; our results agree with animal-growth tests and are in general agreement with the results of Baker, Wright, and Drummond (1937), who have obtained by the bradycardia method values ranging from 3.6 to 7.8 micrograms per gram on six wheats.

The vitamin B_1 content of various kinds of wheat and the influence of climatic conditions on the vitamin content have not been studied. We believe such an investigation would be well worth while.

Three flour mills have kindly supplied us with samples from their mill streams for the purpose of this study. We are greatly indebted to Mr. Maveety of the National Biscuit Company for one of the samples.

Table I is self-explanatory. The summation in the last column is approximately 10% in excess of the analysis of the original wheat. We do not believe that this discrepancy affects the significance of the data; on the contrary, in view of the possible errors in the sampling of the mill stream and other errors, the agreement is surprisingly good.

TABLE I

MILL STREAM ANALYSIS—MILL A

	Percent of wheat	Thiamin per gram	Thiamin 1 g. wheat
	%	γ	γ
Wheat	100	6.25*	—
Bran	13	16.0	2.08
Middlings (shorts?)	13	28.0	3.64
Low-grade flour	7.1	4.5	0.319
Bakery flour	64.2	0.85	0.546
Ground Screenings	2.7	6.4	0.173
			6.758*

In Table II we have a somewhat more extensive picture, although the original wheat was unavailable. It is interesting to note that a summation (not in table) of the bran, shorts, and straight flour gives 5.9 gammas per gram for the original wheat, which is a reasonable figure. From the table can be seen the relation between straight, long-patent, and short-patent flour. The summation in the last column in this case shows a very good agreement.

TABLE II

MILL STREAM ANALYSIS—MILL B

	Percent of wheat	Percent of straight flour	Thiamin per gram	Thiamin 1 g. straight flour
	%	%	γ	γ
Bran	13.75	—	13.3	—
Shorts	13.75	—	21.0	—
Straight flour	72.5	100	1.5*	—
Short patent	—	73	0.7	0.51
Fancy clear	—	23	2.7	0.62
Low grade	—	4	10.3	0.41
Long patent	—	90	1.2	—
				1.54*

The mill stream from Mill C provides the most complete picture. Part I indicates the major distribution of the thiamin of the wheat berry. This mill is the only one of the three which milled out the germ. The summation of the wheat fractions shows very good agreement in this case. It is interesting to note that the richest fraction of wheat (the germ) actually contains only 5% of the original thiamin. As the wheat berry contains about 1½% to 2% of germ, it is probable

TABLE III

MILL STREAM ANALYSIS—MILL C, PART I

	Percent of wheat	Thiamin per gram	Thiamin 1 g. wheat	Percent total thiamin
	%	γ	γ	%
Wheat	100	5.70*	—	—
Germ	1	30.0	0.3	5.0
Bran	17	13.2	2.24	37.8
Shorts	10	23.0	2.3	38.8
Straight flour	72	1.5	1.08	18.0
			5.92*	

that the germ accounts for 10% of the thiamin of the wheat berry. In milling practice not all the germ is recovered. Fully 82% of the thiamin finds its way into feeds, and Table IV shows how the remainder of the thiamin is distributed on further refining.

TABLE IV

MILL STREAM ANALYSIS—MILL C, PART II

	Percent of straight flour	Thiamin per gram	Thiamin 1 g. straight flour	Percent thiamin in straight flour
	%	γ	γ	%
Straight	100	1.5*	—	—
Short patent	80	0.7	0.56	41.8
First clear	12	1.8	0.216	16.6
Second clear	7	5.1	0.35	26.6
Red dog	1	21.2	0.212	21.2
First mids	—	0.3	—	—
Third break	—	0.6	—	—
			1.338*	

As may be seen from the last column, more than 50% of the remaining thiamin is removed in the process, which yields a short patent flour from a straight flour. The summation of the fractions which come from the straight flour shows a reasonable agreement.

Included in this table are thiamin assays on two special flours not usually available. The first mids flour, which has the very low thiamin content of 0.3 microgram per gram, represents the best 33% of the short-patent flour and is probably an example of a highly refined flour. The third-break flour (which constitutes about 3½% of the short-patent flour) is also low in thiamin content (0.6 microgram per gram).

The general trend of our data would seem to indicate that the more

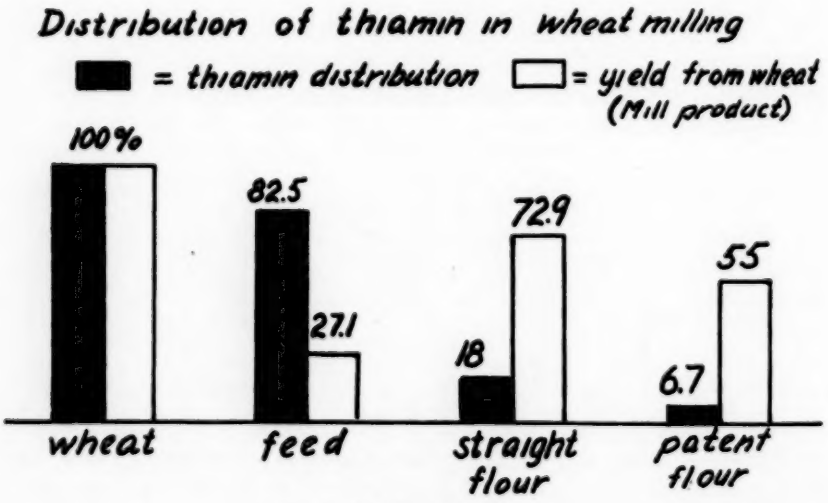

Fig. 1. Distribution of thiamin.

highly refined a flour is the lower will its thiamin content be. Figure 1 summarizes our somewhat limited data.

The bars marked "feed" represent the combined results on bran, shorts, and germ.

What is the relation of these studies to the vitamin content of bread? The following statement of Williams and Spies (1938) from their recent book on vitamin B_1 is of interest:

"In yeast breads there appears to be fairly general agreement that the thiamin content, on an air-dry basis, corresponds very closely to that of the flours used."

In general we find this statement approximately true. The recent advent of a high-vitamin baker's yeast provides, of course, an outstanding exception. The thiamin content of this yeast is so high that it is possible for the baker, using 2% of this yeast based on the flour, to produce a fine white loaf to which has been restored practically all of the thiamin removed in the milling process.

Table V shows thiamin assays made on a series of commercial white breads and whole wheat breads. Included for comparison are values for white bread made with high vitamin yeast.

TABLE V

THIAMIN IN BREAD

	Thiamin per one-pound loaf
	γ
White bread	410; 321; 456
Whole wheat bread	1760; 1620
High B, white bread	1650; 1750

Thus it may be seen that the fermentation method applied to milling and baking technology is a useful and reliable tool.

Summary

The vitamin B_1 content of wheat, flour, and bread has been determined by the fermentation method.

The straight flours analyzed had 1.5 micrograms of thiamin per gram and the short patent 0.7 microgram per gram.

Several mill streams have been analyzed for thiamin content and it has been found that the more highly refined a flour is the less thiamin it is likely to contain.

The thiamin content of air-dried bread roughly corresponds to the thiamin content of the flour except when a special yeast has been used. When a high-vitamin yeast has been employed a white bread may contain as much thiamin as a whole wheat bread.

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FERMENTATION OF MALTOSE IN THE DOUGH

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(Read at the Annual Meeting, May 1939)

Upon completion of recent studies of maltose fermentation (Schultz and Atkin, 1939) it was thought desirable to extend the investigation to fermentation in the dough. Although flour contains but little maltose compared with about 1% of sucrose, the formation of a dough initiates diastatic activity and the maltose thus produced is the principal sugar involved in the later stages of fermentation.

The rate of fermentation of this complex system may be considered from two angles. First, we can study those factors which may affect fermentation in general and, second, those factors which affect the fermentation of a specific sugar (in this case, maltose). Thiamin, under appropriate conditions, causes a most pronounced increase in the rate of fermentation (Schultz, Atkin, and Frey, 1937, 1938). However, although flour is relatively low in thiamin content, the proportion of flour to yeast is usually so high that the fermentation rate of a dough can seldom be increased by added thiamin. In other words, thiamin is not a limiting factor in the presence of sufficient flour.

The stimulation of fermentation by thiamin is dependent on the presence of ammonia nitrogen or of amino nitrogen in the fermenting medium. Without nitrogen in such form thiamin gives but a slight stimulation and consequently it must be concluded that nitrogen (in proper form) is also a potent stimulator of fermentation. Flour is not unduly rich in this fermentation factor, and as will be shown later it has been found that the fermentation rate of a flour-yeast-maltose-water mixture may be affected by added nitrogen.

Maltose fermentation differs from dextrose fermentation in that it is attended by a considerable induction period which has been described by Blish and Sandstedt (1937). As has been reported elsewhere (Schultz and Atkin, 1939) the addition of a small quantity of dextrose, sucrose, etc., remarkably shortens the induction period. It was also shown that extracts containing maltase, such as a dried yeast extract,

have a similar effect. Since flour contains about 1% of sucrose it is logical to suppose that there will be no induction period of consequence in a flour-yeast-maltose-water system.

Recently Sandstedt and Blish (1938) have attempted to explain differences between flours in the third-hour or "proof time" fermentation rates. The explanation was offered that the flours differed in "activator" content.

If the fermentation rate of such mixtures is examined in the light of the foregoing résumé it will at once be seen that the amino-nitrogen factor must be eliminated before the existence of a specific maltose fermentation factor can be postulated.

Experimental

The experimental procedure employed was patterned after that used by Sandstedt and Blish with the exception that the gas evolved was measured in gasometers at atmospheric pressure in a manner initially described by Schultz and Landis (1932). To a reaction bottle which contained 20 g. of flour and 0.8 g. of maltose 20 ml. of H_2O was added in which was suspended 0.6 g. of yeast. The mixture was stirred until uniform and then placed in the machine at 30° C. Readings may be taken at any time but for the present purpose only the gas produced during the third hour was recorded. When other substances were included in the mixture they were weighed directly into the bottles or incorporated in a portion of the water.

Employing a high-grade patent flour, we tested four different forms of nitrogen and Table I gives the results. The stimulations obtained

TABLE I

EFFECT OF ADDED NITROGEN ON THE THIRD-HOUR FERMENTATION RATE OF A YEAST-FLOUR-MALTOSE-WATER MIXTURE MADE WITH A PATENT FLOUR

Addition	Ml. gas produced during third hour
None	109
50 mg. ammonium sulphate	134
50 mg. carbamide	121
50 mg. asparagin	140
50 mg. aspartic acid	125

make it quite apparent that nitrogen may be responsible for a significant part of the differences between flours. The flour with which these tests were made does not show the high response which may be observed with certain other flours. Table II shows the response obtained with a series of three flours. In the absence of added nitrogen a considerable difference may exist in the third-hour fermentation rate. The addition of ammonia nitrogen makes the rates nearly equal. The first mids flour, however, did not quite equal the rate of the other two

TABLE II

FERMENTATION RATE OF DIFFERENT TYPES OF FLOUR

Flour	Ml. gas produced during third hour		
	No addition	With added nitrogen	With added nitrogen and thiamin
Straight	110	147	No increase
Short patent	99	143	—
First mids	69	132	+12 ml.

when nitrogen was added. As may be observed in the last column the deficiency was due to lack of thiamin. This correlates with thiamin assays of the three flours, which showed 1.5, 0.7, and 0.3 gamma per gram respectively for the straight, patent, and first mids.

Although the patent flour with which most of our tests were made showed an intermediate response to nitrogen it was thought advisable to make our principal studies on that flour since it more nearly represents an average flour.

It has been known for a long time that amino acids occur in flour. Blish (1918) reported that "normal patent flour contains about 2 mg. of amino-acid nitrogen for every 100 g. of flour, and about three times as much nitrogen in free acid amide form." This is a clear indication that amino nitrogen occurs in flours in amounts which may explain the differences between them. Furthermore, it may be expected that the process of autolysis will sometimes produce further quantities of amino acids by protein breakdown. To see which of the amino acids may be responsible for the increased "proof time" fermentation rate a study of 22 amino acids was made.

In each case the acid was added to the dry flour-maltose mixture and well stirred before addition of the yeast suspension. Of the 22 amino acids tested, 6 showed a stimulating effect, 6 were relatively inactive, and 10 decreased the rate. Table III gives the figures. Since all tests were not made on the same day we have, for purposes of comparison, reduced the control values for the third-hour gas production to 100 and expressed the others on that basis. The quantity of acid added was 50 mg. except for the synthetic acids, of which 100 mg. of the *dl* form was used.

Discussion

Of the significance of the results with specific amino acids in the amounts employed, little can be said at present. The object of this study was merely to show that these substances and perhaps others of similar activity must be considered as possible factors in the difference in "proof time" rate which has been observed with different flours. In general, our results show that numerous factors enter into maltose

TABLE III

INFLUENCE OF AMINO ACIDS ON THE THIRD-HOUR FERMENTATION RATE OF YEAST-FLOUR-MALTOSE-WATER MIXTURES—WITH PATENT FLOUR

Amino acid	Gas production	Amino acid	Gas production
	ml.		*ml.*
l-asparagine	128	*l*-tyrosine	96
d-arginine·HCl	121	*dl*-methionine	95
d-glutamic acid	118	*l*-histidine·HCl	93
dl-valine	117	tryptophane	93
dl-aspartic acid	115	*l*-proline	93
dl-lysine·2HCl	111	*dl*-threonine	92
dl-alanine	104	*dl*-amino butyric acid	88
l-hydroxyproline	102	*l*-leucine	87
l-cystine	102	glycine	83
glutathione	102	*l*-cysteine·HCl	67
dl-phenylalanine	102	*dl*-nor-leucine	65
Control	100	Control	100

fermentation in the dough but that each factor may (with proper care) be separated and studied individually. The actual fermentation of the dough is then the integration of all factors present.

Summary

The fermentation rate of maltose may be limited by the absence of several factors: thiamin, dextrose (or sucrose), and amino nitrogen.

In yeast-flour-maltose-water mixtures, thiamin and sucrose are generally present in sufficient quantity and are not limiting factors.

Experiments with nitrogen (in ammonia or amino form) indicate that differences in the third-hour fermentation rate between various flours may be due to differences in their amino-nitrogen content.

The effect of 22 amino acids on the third-hour fermentation rate of a yeast-flour-maltose-water mixture made with a patent flour is described.

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THE ELECTROMETRIC DETERMINATION OF DIASTATIC POWER OF MALTS[1]

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(Read at the Annual Meeting, May 1939)

The official method of the American Society of Brewing Chemists (1935) for the determination of diastatic power is very time-consuming and is subject to large variations in the values obtained among different laboratories. These variations are probably due to individual differences in technique in carrying out the titration of boiling Fehling's solution. Anderson and Sallans (1937) and Sallans and Anderson (1937) have discussed the shortcomings of this method.

In an attempt to increase the speed with which diastatic power determinations may be completed and to simplify the procedure as much as possible while maintaining reasonable accuracy, the electrometric procedure was tested. Several reagents were tried as well as two electrode systems. The procedure finally adopted is essentially that of Shaffer and Williams (1935) for glucose in biological materials.

Procedure

The malt infusion and starch solution are prepared according to the official method of the A.S.B.C. (1935). The diastasis is carried out as described by Anderson and Sallans (1937) and a blank is run for each determination as in the official method.

The equipment for measuring the reducing power of the digested starch solution electrometrically consists of the following: A potentiometer capable of measuring 0 to 100 millivolts with an accuracy of one-half millivolt, two bright platinum electrodes made by sealing lengths of platinum wire into glass tubes with provision for connecting to the potentiometer and a salt bridge of agar jel saturated with potassium chloride, a boiling-water bath and a 25° C. water bath, 125-cc. Erlenmeyer flasks, a precision 5-cc. pipette for taking samples of digested starch, and a 10-cc. pipette or a burette for measuring the reagent.

The reagent used is Reagent I of Shaffer and Williams (1935). This consists of

29.6271 g. potassium ferricyanide ($K_3Fe(CN)_6$)
4.2233 g. potassium ferrocyanide ($K_4Fe(CN)_6 . 3H_2O$)
50 g. sodium carbonate (Na_2CO_3)

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.
[2] Industrial Fellow under the United States Maltsters Association industrial fellowship grant to the University of Wisconsin. The Federal WPA Natural Science Project at the University of Wisconsin has contributed to the research program.

10 g. sodium bicarbonate ($NaHCO_3$)
117 g. sodium chloride (NaCl)

made up to one liter. Analytical Reagent grade salts are used and the solution need not be standardized.

Determination of Reducing Power

To determine the reducing power of the digested starch 10 cc. of reagent is transferred to a 125-cc. Erlenmeyer flask and 5 cc. of the starch sample is added. A cork is placed loosely in the flask and the flask then placed in the boiling-water bath for exactly 20 minutes. At the end of this time it is placed in the 25° bath and the temperature readjusted to 25° C. after a few minutes.

The electrode system is set up as follows: The reference electrode consists of a small thin-wall vial containing 10 cc. of ferricyanide reagent and 5 cc. of water in which one platinum electrode and one arm of the salt bridge are immersed. The reference electrode is positive. The other arm of the salt bridge and the second platinum electrode are supported in such a manner that they may be inserted in the neck of the 125-cc. Erlenmeyer flask and immersed in the reaction mixture by raising the flask. A beaker (600 cc.) is filled with water from the 25° bath and the reference electrode immersed in it. Thus both electrodes are at 25° during measurement of the potential of the system.

The corresponding starch blank is run in exactly the same manner. The reading in millivolts of the sample minus the reading of the blank gives the E.M.F. value which is used to obtain the final result in degrees Lintner on the existing moisture basis.

Experimental

A series of determinations were run on a sample of pure maltose monohydrate and the resulting E.M.F. values were found to have a straight-line relationship to the amount of maltose present.

Two series of malt samples were then selected, one covering the range of 40 to 140 degrees Lintner and the other 130 to 250 degrees Lintner on the existing moisture basis. The diastatic powers of these malts were carefully determined by the official A.S.B.C. procedure and by the electrometric. Both methods were run on the same partially saccharified starch solutions. The series covering the lower range of diastatic power contained 14 malts. That for the higher range contained 16 samples. These data were then used to calculate the equations of the relationship between degrees Lintner and E.M.F. The relationship is a straight-line function for both ranges of diastatic power.

The equation for samples in which 2 cc. of infusion and 100 cc. of starch are used is $y = 2.734x - 25.49$, where y equals degrees Lintner on the existing moisture basis and x is the E.M.F. For samples in which 1 cc. of infusion and 100 cc. of starch are used $y = 5.155x - 39.96$.

If curves are drawn on large sheets of millimeter graph paper using 1 cm. per millivolt and 1 mm. per degree Lintner, the results may be read off directly within 2 to 3 degrees Lintner when the E.M.F.'s are read to one-half millivolt.

Comparison of Results with Official and Electrometric Methods

In order to compare the two methods eight malts were used. Three malts were in the range below 140 degrees Lintner and consequently were run with 2 cc. of infusion. The other five malts were in the upper range and were run with 1 cc. of infusion. In order to determine the variability of each method, four sets of determinations were run on each malt. The determinations and blanks for each method were carried through in duplicate.

Table IA gives the means of duplicate determinations of reducing power in degrees Lintner and the mean of the four replicate determi-

TABLE I

COMPARISON OF ELECTROMETRIC AND A.S.B.C. METHODS FOR DETERMINING DIASTATIC POWER

Means of Duplicate Determinations of Reducing power in Degrees Lintner and Means of the Four Replicate Runs on Each Malt

TABLE IA—LOW-DIASTATIC MALTS WITH 2 CC. INFUSION

M–1635		M–1643		M–1594	
Elec.	ASBC	Elec.	ASBC	Elec.	ASBC
83.3	88.4	96.0	99.4	136.3	135.2
84.5	85.8	98.5	96.5	141.0	133.8
86.3	86.2	98.5	98.0	140.0	134.5
83.5	84.4	97.0	97.1	138.0	137.5
Mean					
84.38	86.18	97.50	97.74	138.82	135.20

TABLE IB.—HIGH-DIASTATIC MALTS WITH 1 CC. INFUSION

M–1633		M–1639		M–1592		M–1642		M–1644	
Elec.	ASBC	Elec.	ASBC	Elec.	ASBC	Elec.	ASBC	Elec.	ASBC
156.0	160.7	170.0	171.3	180.0	188.8	172.5	173.2	186.3	190.3
159.5	160.8	161.3	170.2	168.3	176.8	170.0	169.5	178.5	182.2
157.0	156.4	161.8	161.8	179.0	184.3	168.5	176.3	180.5	181.3
157.5	159.4	167.5	163.6	176.0	178.6	169.0	174.3	184.0	188.5
Mean									
157.50	159.32	165.15	166.72	175.82	182.12	170.00	173.32	182.32	185.57

nations for each of the malts run with 2 cc. of infusion using the two methods. Table IB gives the same data for the malts run with 1 cc. of infusion.

Tables II and III give the analysis of variance on the values in degrees Lintner obtained with the two methods using 2 and 1 cc. of infusion.

TABLE II

ANALYSIS OF VARIANCE ON THE DIASTATIC POWER VALUES IN DEGREES LINTNER OBTAINED BY BOTH METHODS WITH 2 CC. INFUSION

Source	Degrees of freedom	Variance	F value	5%	1%
Malts	2	11,679.65	2,369.1	3.55	6.01
Methods	1	3.31	0.67	4.41	8.28
Malts x methods	2	31.04	6.30	3.55	6.01
Exp. error	18	4.93	5.94	2.08	2.85
Dup. error	24	0.83	—	—	—

It will be noted in Table II that the "F value" for interaction of malts and methods is significant but not extremely so. This is probably due to the fact that the two malts with the lower diastatic powers in Table IA are slightly lower when measured by the electrometric method while the third malt runs somewhat high by the electrometric method as compared with the A.S.B.C. procedure. Also the third malt lies very close to the upper limit in diastatic power for which 2 cc. of infusion can be safely used. In Table III the "F value" for the interaction of malts and methods is quite insignificant.

TABLE III

ANALYSIS OF VARIANCE ON THE DIASTATIC POWER VALUES IN DEGREES LINTNER OBTAINED BY BOTH METHODS WITH 1 CC. INFUSION

Source	Degrees of freedom	Variance	F value	5%	1%
Malts	4	1,650.16	55.47	2.69	4.02
Methods	1	212.55	7.14	4.17	7.56
Malts x methods	4	14.15	0.48	2.69	4.02
Exp. error	30	29.75	6.30	1.72	2.20
Dup. error	40	4.72	—	—	—

Also in Tables II and III the error variance between duplicates is much higher for the malts run with 1 cc. of infusion. This is probably due to the increased error in pipetting 1 cc. of infusion although precision-grade pipettes were used and care taken in the experimental procedure. A number of determinations were run with 2 cc. of infusion

and 200 cc. of starch. These data are not included here but there was distinctly less variation between replicates than when 1 cc. was used.

TABLE IV

ANALYSIS OF VARIANCE TO DETERMINE ERROR VARIANCE AND COMPARISON OF PRECISION OF THE TWO METHODS WITH 2 CC. INFUSION

Method	Error variance	F value	5%	1%
Electrometric	5.22	1.13	3.20	5.33
A.S.B.C.	4.63	—	—	—

TABLE V

ANALYSIS OF VARIANCE TO DETERMINE ERROR VARIANCE AND COMPARISON OF PRECISION OF THE TWO METHODS WITH 1 CC. INFUSION

Method	Error variance	F value	5%	1%
Electrometric	25.73	1.31	2.43	3.58
A.S.B.C.	33.78	—	—	—

A comparison of the error variances of the two methods when 1 and 2 cc. of infusion are used is given in Tables IV and V. Where 2 cc. of infusion is used the error variance of the A.S.B.C. method is lower than that of the electrometric method. However, in the series where 1 cc. of infusion is used the electrometric error variance is lower than the A.S.B.C. In both cases the ratios of the error variances give "F values" below the 5% level of significance. This would indicate that there is no important difference in precision between the two methods.

Summary

The electrometric procedure is simple and requires no great technical skill. Only one reagent is required, which need not be standardized if pure salts are used in its preparation and the ferri- and ferrocyanides accurately weighed out. Any simple potentiometer is satisfactory provided it covers the necessary E.M.F. range. A modified quinhydrone pH meter was used for a large number of routine determinations and while not as accurate as a Leeds and Northrup potentiometer it gave sufficiently accurate results for ordinary routine work. The electrometric procedure is very rapid. One operator can determine the reducing powers of twelve samples including blanks within two hours or less after the enzyme hydrolysis is complete, whereas only four to six samples could be completed in the same time with the A.S.B.C. procedure.

The electrometric method compares favorably with the A.S.B.C. method and is sufficiently precise for use with routine samples.

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A COMPARISON OF METHODS FOR THE DETERMINATION OF DIASTATIC POWER OF MALTS

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Considerable interest has recently been evidenced in the development of a method for the determination of the diastatic power of malt which from the standpoints of precision and rapidity will prove superior to the Official Method of the American Society of Brewing Chemists (1936). Anderson and Sallans (1937) have applied Blish and Sandstedt's (1933) ferricyanide method for flours to the determination of diastatic power of malts and show that it is much more rapid and somewhat more precise than the A.S.B.C. method. Hildebrand and McClellan (1938) suggested the ceric sulfate titration as a desirable modification of the original Blish and Sandstedt method.

This paper represents a brief report on a comparison of the precision of the A.S.B.C. method, Anderson and Sallans' modification, and the ceric sulfate titration applied to the determination of the diastatic power of malts.

Methods

Seven malts with different diastatic powers were used and in order to determine the variability within each method, six determinations on each malt were made with each method. The official procedure was used throughout, up to the determination of the reducing power of the digested starch solution, with the exception that two milliliters of the undiluted malt infusion were used in all determinations. For each run a single extraction of the malt and digestion of the starch solution was made, including the blank, and the quantity of reducing materials in the same solutions was determined in duplicate by each

[1] Cooperative investigations between the U. S. Department of Agriculture, Division of Cereal Crops and Diseases, Bureau of Plant Industry, and the University of Wisconsin.

method. The values were then calculated in milligrams of maltose produced by 2 milliliters of infusion. The Lintner value was calculated for the A.S.B.C. method in the usual way and this was multiplied by the ratio of milligrams of maltose by other methods to the milligrams of maltose calculated for the A.S.B.C. method, to obtain values in degrees Lintner for the Anderson and Hildebrand methods. At frequent intervals, the solutions were standardized against a purified dry sample of maltose and the maltose equivalents of the various solutions used in the calculations.

Presentation and Discussion of Data

Table I contains the means with their standard errors of the six duplicate determinations on each malt and with each method.

TABLE I

MEANS OF SIX DUPLICATE DETERMINATIONS, WITH THEIR STANDARD ERRORS, OF DIASTATIC POWER ON SEVEN MALTS IN MILLIGRAMS OF MALTOSE PRODUCED BY 2 ML. MALT INFUSION AND IN DEGREES LINTNER

Malt No.	Milligrams maltose			Degrees Lintner		
	Anderson	A.S.B.C.	Hildebrand	Anderson	A.S.B.C.	Hildebrand
1	371± 3.2	366± 3.3	398± 5.3	99±0.8	97±1.0	106±1.5
2	510± 4.0	509± 3.4	557± 9.1	136±1.1	136±0.8	148±2.7
3	517± 9.1	523±11.6	577± 3.5	138±2.3	140±2.9	154±1.7
4	580± 7.3	591± 5.0	633± 9.2	154±1.8	158±1.5	169±2.4
5	619± 6.0	619±11.5	672±14.9	165±1.9	165±3.2	179±4.0
6	688±14.2	690±14.2	747±16.8	184±3.7	184±3.9	199±4.3
7	720± 8.1	716±14.5	729±10.5	192±2.4	191±3.7	195±3.0

An analysis of variance was calculated on the data from all three methods and significant F values were obtained for methods and for the interaction of malts with methods. However, when the analysis was applied to data from the A.S.B.C. and Anderson methods only, these values were not significant. Hence the high values for diastatic power obtained with the Hildebrand procedure appeared to be responsible for the significant F values for methods. These high values were consistent for six of the malts, but on the seventh the results agreed well with the other two methods and this probably accounts for the high interaction value for malts and methods.

The variation within the six determinations on each malt, as shown by the standard errors, fluctuates considerably with the different methods and on the different malts, but is only slightly in favor of the Anderson method.

In order to compare the precision of the three methods, analysis of variance was calculated on the values obtained with each method

on all of the malts. The condensed statistics from the calculation using values in degrees Lintner are given in Table II. From this it is seen that the Anderson method has the lowest error variance, being somewhat the most precise of the three. However the comparisons give F values which do not exceed the 5% level of significance, so there is no essential difference in precision between the three methods. The F value obtained in comparing the Anderson and Hildebrand methods equals the 5% point and might become significant if more malts were used.

TABLE II

COMPARISON OF PRECISION OF THREE METHODS ON ALL OF THE MALTS USING VALUES IN DEGREES LINTNER

Method	Error variance	Comparison	F value	5%	1%
Anderson	4.71	Anderson and Hildebrand	1.77	1.77	2.24
A.S.B.C.	7.29	A.S.B.C. and Anderson	1.55	1.77	2.24
Hildebrand	8.35	Hildebrand and A.S.B.C.	1.14	1.77	2.24

Because of the high values and the somewhat erratic results obtained with the Hildebrand method, a further study of this method was undertaken. Comparable results on malt 7 (Table I) were obtained with a new ceric sulfate solution which differed somewhat in concentration from the first solution used. The effect of concentration on the oxidizing power of ceric sulfate solutions has been checked, and no significant effect found within the rather narrow limits used.

In order to determine whether slight errors in the preparation of the solutions might be responsible for the high results, four new stock solutions of ceric sulfate were made up and diluted to 0.0176 normal. Also four new potassium ferricyanide solutions were prepared. These solutions were used in various combinations in determining the diastatic power of seven additional malts. Table III shows the means of two determinations on each malt using the combinations of solutions shown, with the Hildebrand method, and the value obtained with the A.S.B.C. method.

While certain combinations of solutions, for example ferricyanide No. 3 with ceric sulfate No. 4, seem to give consistently higher results than the others, the differences are not great and do not seem to indicate that the high values obtained in the earlier work were due to such a cause. Also in this series of determinations all of the values obtained with the Hildebrand method were appreciably higher than those obtained with the A.S.B.C. method. As in the earlier study the difference between ceric sulfate and A.S.B.C. values varied for different

malts, making the use of a correction factor unsatisfactory. The cause of the high values and erratic behavior of the ceric sulfate method is still unexplained.

TABLE III

MEANS OF TWO DETERMINATIONS OF DIASTATIC POWER IN DEGREES LINTNER ON SEVEN MALTS USING DIFFERENT COMBINATIONS OF REAGENTS FOR THE HILDEBRAND METHOD

Combination of reagents		Malt numbers					Combination of reagents		Malt numbers	
Ferricyanide solution no.	Ceric sulfate solution no.[1]	8	9	10	11	12	Ferricyanide solution no.	Ceric sulfate solution no.[1]	13	14
3	3	95	110	129	146	187	1	1	150	177
4	3	95	109	129	145	186	2	1	152	180
3	4	98	112	132	148	190	1	2	151	180
4	4	95	110	128	145	183	2	2	157	182
Value with A.S.B.C. method		85	100	115	132	167	A.S.B.C. value		145	172

[1] The ceric sulfate solutions used here were all 0.0176 N.

Conclusions

On the limited number of malts used in this study, the A.S.B.C. and Anderson methods of determining diastatic power gave values which were very close. The Hildebrand method for some unexplained reason gave erratic results, these being consistently high on six of the malts and agreeing very well on one. Further study failed to find the cause of this disagreement.

There are no significant differences in precision in the three methods, the Anderson method being only slightly more precise than the other two. However, its appreciable advantage in rapidity over the A.S.B.C. method as discussed by Anderson, gives it more value for routine analytical determinations.

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SOME FACTORS INFLUENCING THE VISCOSITY OF RICE FLOUR SUSPENSIONS

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Pure rice flour is used today as a filler and thickener for many prepared foods. As such its viscosity characteristics in water solution become highly important, especially when the product is a canned food which must be subjected to high pressures and temperatures for sterilization. This investigation was conducted for the purpose of determining the factors responsible for the variation in viscosity of rice flours.

Material and Methods

Rice flour ground from broken kernels of brewer's rice was employed in these tests. The viscosity apparatus was the Bauer Viscosimeter (Fig. 1). This is a machine having a constant-speed motor turning two paddles immersed in the flour-and-water suspension. The resistance offered to these paddles is registered through a differential on a chart moving at constant speed. The suspension bowl is set in a jacket. By this means, the temperature of the suspension may be controlled from 15° C. to 100° C. by flowing water or steam through the jacket. This instrument is sensitive enough to measure changes of 1% in a glucose solution.

For these tests, one part rice flour to 6.5 parts of water was used. Steam was introduced into the jacket at a constant rate of speed for all tests; the rate of rise in temperature to the gelatinization point was the same in all cases. The viscosity, as demonstrated by the curve, rose as the heat swelled the starch granules. Maximum consistency was attained at 82°–85° C., the gelatinization point. After this, the curve dropped to an average point which it held as long as the temperature was kept constant. Variation in the temperature then brought about changes in the viscosity. Figure 2 shows a typical chart obtained with this apparatus.

A correlation between the high points of the curves and viscosity of rice-flour suspension during and after a canning process was observed. Those flours having the highest maximum points in viscosity as measured by the Bauer instrument had the greatest consistency after the canning process. Therefore, comparison of high points of viscosity curves offered a good method for determining the quality of the flour.

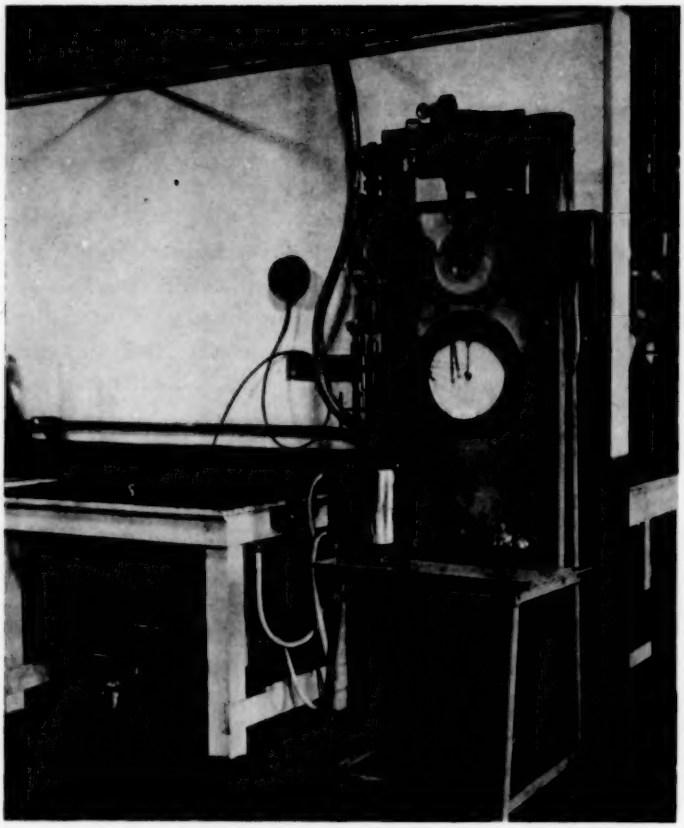

Fig. 1. The Bauer Viscosimeter.

Effect of Particle Size upon Viscosity

The effect that small or large granules have upon the consistency of rice flour suspensions was first determined.

TABLE I

RICE FLOUR VISCOSITY AS RELATED TO PARTICLE SIZE

Sample No.	Screen	Viscosity
126	On 60 M.	48
126	Through 60 M.	56
1433–R mill	On 80 M.	66
1433–B mill	On 100 M.	72

These results seem to indicate that the finer granulation of the starch particles permits greater water absorption and results in greater viscosity.

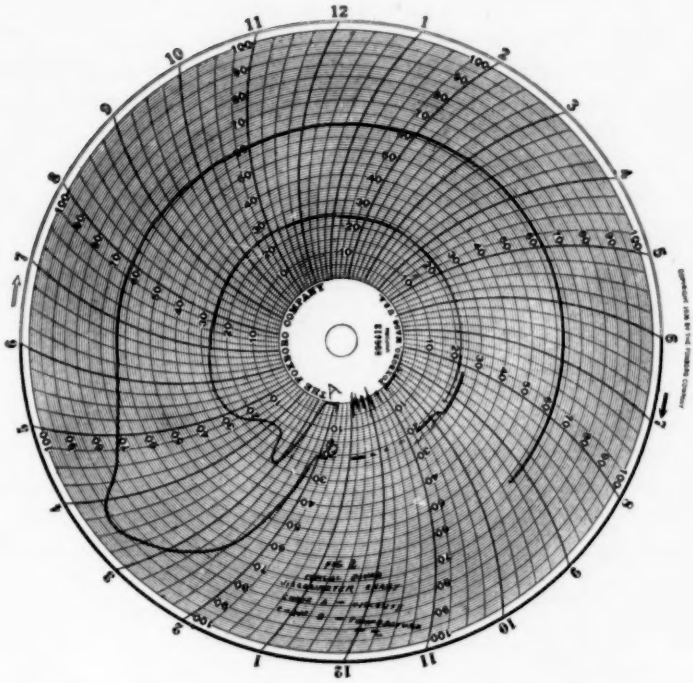

Fig. 2. Typical viscosimeter chart. Curve A, viscosity; Curve B, temperature in Centigrade degrees.

Effect of pH on Viscosity of Rice Flour

Since it is an accepted fact with authorities in starch chemistry that acid reduces viscosity and alkalies increase the viscosity of starch suspension, pH was determined, electrometrically, on a number of samples in conjunction with viscosity curves.

The figures from Table II seem to indicate that viscosity tends to follow pH. Low pH levels give low viscosities, while in a general

TABLE II

Relation of pH to Viscosity of Rice Flour Suspensions

Sample No.	Viscosity	pH
1	66	5.5
2	55	6.1
3	57	6.2
4	67	6.3
5	68	6.35
6	72	6.3
7	72	6.35
8	79	6.9
9	82	6.9
10 (pure rice starch)	87	6.95

way high viscosities were obtained at high pH levels. Similarly, it was observed that those flours which had high viscosity curves with a pH close to neutral had the greatest consistency when subjected to a canning process. The critical pH range in which greatest changes in viscosity were noted was 6.4–6.9.

Factors Influencing pH of Rice Flour

Winton found that rice oil increases in acidity with great rapidity on aging. He also found that this oil contained at times as much as 90% free fatty acids.[1]

Ash and fat were determined on several samples of rice flour by the A.A.C.C. method. The fat was titrated with *n*/10 NaOH using phenolphthalein.

TABLE III

RELATION OF FAT ACIDITY TO pH AND VISCOSITY OF RICE FLOUR

Sample No.	Ash	Fat	Neutralized by 1 g. fat	Viscosity	pH
	%	%	*cc. n/10 NaOH*		
1	0.896	0.75	26.7	66	5.5
2	0.937	1.25	23.3	55	6.2
6	0.898	1.37	21.8	72	6.3
7	0.561	0.88	22.5	72	6.35
9	1.4	1.46	4.5	82	6.9

Quantity of fat, as such, does not seem to affect the viscosity or the pH. However, the amount of free fatty acid present, as shown by the NaOH neutralization figure, seems to have a bearing on the pH of the flour. Compare Nos. 1 with 9, and 1 with 6.

TABLE IV

CONSTITUENTS OF RICE ASH

(These are average figures.)

P_2O_5	SO_3	SiO_2	Cl	Na_2O	K_2O	CaO	MgO	Fe_2O_3
52.1%	0.6%	3.1%	0.09%	5.3%	22.1%	3.4%	11.7%	1.6%

Constituents in the ash also seem to affect the pH. Where the ash percentage is high, the potassium hydroxide and phosphates contained therein can buffer the free fatty acids of the fat. Since these two alkali- and acid-producing compounds are present in varying amounts in different flours, the degree of buffering would also vary. This would give rise to varying degrees and quantities of acidity in the flour.

[1] A. L. Winton and K. B. Winton, The structure and composition of foods, Vol. I, p. 146, Wiley and Sons, N. Y., 1932.

It was also found that rice which had been stored longest, even under normal dry conditions, when ground into flour showed the lowest pH and the lowest viscosity. Further investigation showed that those rice lots which were low in ash and fat to begin with, denoting the removal of almost all of the bran, showed the least change in pH on storage. Apparently the low oil content was responsible for this. Storage of two to three months seemed sufficient to begin deterioration of the fat with the liberation of fatty acids.

Summary

Two factors seem to affect the viscosity of rice flour. They are granule size and pH. The latter seems to be regulated in turn by the amount of free fatty acids present. Rice oil becomes rancid very readily on storage, thus accounting for the free fatty acids. Quantity and composition of the ash apparently are factors in the buffering of these acids. Rice, high in fat when stored for several months' time, has a lowered viscosity, quite possibly due to increase of fatty acids.

MEASURING FERMENTATION RATE AND GAS LOSSES IN DOUGH [1]

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The device first described by Bailey and Weigley (1922) and substantially redesigned by Bailey and Johnson (1924) for measuring the rate of expansion of, and the CO_2 loses from, a fermenting flour dough has passed through a process of evolution which has resulted in substantial changes since it was last described. Since it has proved singularly useful in our hands and in other laboratories in a variety of connections, it appears desirable to publish the necessary diagrams and descriptions so that it may be made available to those who may find use for it.

In its present and latest form, a heavy copper cylinder 8 cm. in diameter and 20 cm. high, shown at *A* in Figure 1, has been substituted for the glass jar used by Bailey and Johnson (1924). This metal container has several advantages over glass. In addition to the fact that it is not liable to breakage, the copper jar is a better conductor of heat than glass, and hence it, and its contents, reach the temperature of the

[1] Paper No. 1703, Scientific Journal Series, Minnesota Agricultural Experiment Station.

thermostat more promptly. It is enough heavier than glass so that there is less difficulty in keeping it submerged in the water thermostat. Moreover it is so much more sturdy than glass that greater force can be applied in seating the lid *B* against the rubber gasket *C* in closing the jar. If necessary, the copper vessel can be held in a clamp or vice while a wrench is used to grasp the square knob in the center of the lid and thus rotate the latter to hermetically seal the vessel. This is often important in insuring a gas-tight joint at the top of the vessel, without which serious leakage may ensue that vitiates the results of the tests.

It will be observed that there is a shoulder near the top of this copper vessel which tapers to a brazed joint with the threaded brass fitting, that constitutes the actual top of the jar. This fitting, 6 cm. in diameter, is of fairly heavy brass, and is of such dimensions that the ordinary mason jar ring may be used as a gasket (*C*). These rings are readily available at a modest price. The lid is threaded into the top and is, in reality, a standard brass plug that can be purchased from a plumbing supply house.

Dough that is under observation is held in a cylindrical glass beaker shown at *F* in Figure 1. This beaker is 5 cm. in internal diameter by 11½ cm. high, and is provided with numerous openings about 5 mm. in diameter distributed fairly evenly over the upper 60% of the side-wall surface. The purpose of these openings is to afford opportunity for the CO_2 which leaks from the dough to move out of the beaker and into the atmosphere of the copper vessel, whence it may be absorbed in strong NaOH solution, used in certain studies to be described later.

In most of the studies of dough with which we have been concerned, a portion equivalent to 40 g. of flour has been found most convenient. This actually involves a quantity of hard wheat flour dough weighing 65 to 70 g., the quantity depending upon the absorption or proportion of water to flour, and the weight of other dough ingredients such as yeast, salt, sugar, shortening, etc. Larger quantities of dough up to the equivalent of 50 g. of flour may be employed in the instance of weaker flours, if such doughs, when fermented, do not overflow the glass beaker. Smaller or larger quantities of dough could doubtless be treated in like manner by an appropriate adaptation of the size of the apparatus.

Two major types of measurements have been made with this device: (1) a direct measurement of the rate of dough expansion and (2) an indirect measurement of the loss of gas from the dough. In the first instance about one-quarter of the space between the glass beaker and the copper vessel up to the level of the lowest row of openings in the side wall of the beaker is filled with 23% sodium chloride. This solu-

tion has an aqueous vapor pressure sufficiently similar to that of an ordinary dough so that the latter does not dry and become crusted over during the period of observation. Moreover it is less likely to absorb CO_2 from the atmosphere of the cylinder than is distilled water.

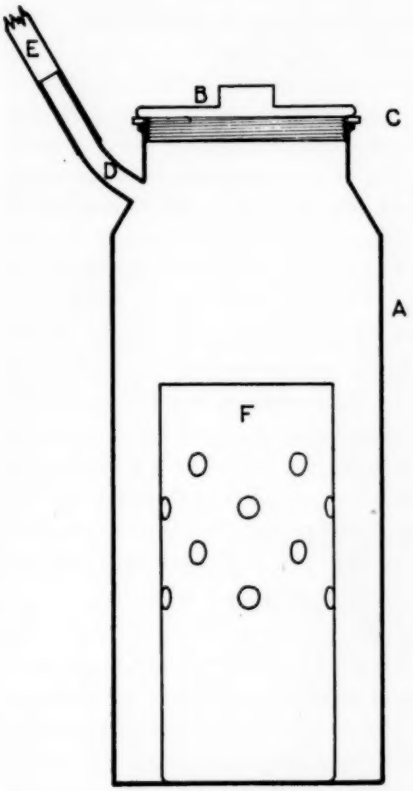

Figure 1.

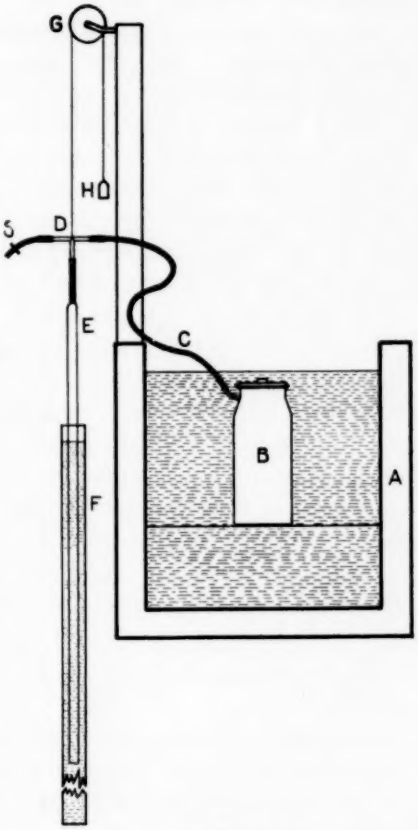

Figure 2.

Fig. 1. Cross section of the copper vessel (*A*), provided with a threaded brass lid (*B*) tightly seated against a rubber gasket (*C*), with a tubulature (*D*) connected with heavy-walled rubber tubing (*E*) which discharges the displaced air into a gasometer, and a perforated glass beaker (*F*) which contains the dough under observation.

Fig. 2. Cross section of the entire gas-measuring system, with a copper lined tank (*A*) filled with water at constant temperature in which the dough-containing vessel (*B*) is submerged, heavy-walled rubber tubing (*C*) connected through a T-tube (*D*) to the burette (*E*) of the gasometer which, in turn, is within the larger tube (*F*) filled with saturated salt solution, a stop-cock or pinch-cock (*S*) to evacuate the gasometer, a pulley (*G*) and a counter weight (*H*) to support the gasometer at the desired levels.

In the second type of measurement mentioned above, an equal quantity of 23% KOH solution is placed in the space indicated. This quickly absorbs the CO_2 which leaks from the dough. The efficiency can be enhanced by providing a blotting-paper cylinder which fits loosely inside of the copper vessel and thus surrounds the side walls of

the beaker containing the dough but does not cover the top. This blotting-paper cylinder functions as a wick to draw the strong KOH solution closer to the ports in the side of the glass beaker, thus reducing the distance in space through which the CO_2 must diffuse before encountering a surface moistened with the absorbent. Since blotting paper such as is used in seed germination tests is suitable for this purpose, and is relatively cheap, it is recommended that a fresh cylinder be used for each test.

As a matter of fact, our experience with the device has indicated that CO_2 is absorbed so promptly in the absence of the paper cylinder that it is probably unnecessary to use it for ordinary work. It was indicated above that the volume of CO_2 which escapes from the dough is actually measured indirectly. Thus the changes in displacement of a dough plus the volume of escaped CO_2 are recorded in the first vessel, which contains no CO_2 absorbent. The change in displacement of the dough is observed in another aliquot of the same dough contained in the second vessel which is charged with the CO_2-absorbent KOH solution. The difference between these two observations represents the volume of CO_2 which escapes from the dough in the second vessel. Such losses of CO_2 from fermenting dough are particularly significant in studies of flour strength, fermentation period, the effect of various flour and dough treatments, and a multitude of other variables involved in flour and dough investigations. These measurements of CO_2 retention are not afforded by most of the other devices proposed for use in determining the fermentation rate of doughs.

A tubulature shown at *D* in Figure 1 is provided to connect the space within the copper vessel to an external gasometer which measures the changes in dough displacement and gas loss. While various types of gasometer, simple and recording, are available, the one used chiefly by us consists merely of an inverted burette (*E* in Figure 2). It is connected with the tubulature of the copper vessel through a glass or metal T-tube (*D*) and heavy-walled rubber tubing (*C*). A suitable pinch-cock or stop-cock is provided (*S*) on the opposite side of the T-tube for use in adjusting initial pressures in the system, and for venting the contents of the burette in returning it to a zero setting.

The burette in question is partially submerged in a glass tube (*F* in Figure 2) which is about twice as large in diameter as the burette, and a little longer than the latter. This glass tube is closed at its lower end, and is filled nearly to the top with aqueous saturated NaCl solution. The saline solution is used in preference to water because CO_2 is less soluble in it. As air is displaced from the copper vessel *B* and passes into the gasometer burette *E*, salt solution is forced down-

ward in the latter and out into the glass tube *F*. Periodically the burette *E* can be elevated to level the liquid within and without. A cord attached to the burette passes over the pulley *G* and a counter weight *H* serves to maintain the burette in the position to which it is manually adjusted.

Since the total volume of air displaced from the dough container may, and doubtless will, exceed the capacity of the gasometer burette, the air accumulated in the latter can be vented periodically by opening the pinch-cock at *S*, the burette returned to a zero setting, and the system again sealed at *S*. The routine here described obviously serves to maintain the air in the dough container at, or very close to, atmospheric pressure, which is the level of pressure under which it is normally fermented in practice. While an automatic gasometer has certain advantages, such devices are much more complicated and in general are more liable to the leakage which may proceed unsuspected and thus give rise to incorrect records. Moreover they are much more expensive, especially when provided with kymographs. When the volume of work is small, such automatic units may be justifiable on the score of conserving the operator's time. With a large volume of work involving a dozen or more doughs the operator finds his time is practically occupied with this one operation, however, and under such circumstances the necessary attention to the periodic adjustment and reading of the burettes can be given incidentally to the general program of fermentation studies. Moreover any leakage or other faults in operation of the device may be promptly detected under this system of manual control with a consequent reduction in the proportion of faulty determinations.

Since the dough and the air in the copper dough-container must be maintained at a known and controlled temperature throughout the period of observation, provision is made for immersing this copper vessel in a water thermostat. While an air thermostat might suffice, it is usually easier to maintain a constant temperature in a water bath. In this laboratory the water thermostat consists of a long narrow cyprus box shown at *A* in Figure 2 in cross section. It is long enough to accommodate 12 copper vessels. The box is lined with copper, and a false bottom of this metal is provided as shown in the diagram, which is about one-third of the distance between the bottom and top of the tank. A circulating pump is provided which drives the water briskly along the bottom of the bath from right to left. At the left-hand end a slot is provided in the false bottom, not shown in the diagram, through which the circulating water may rise from the lower to the upper compartment. Thence it continues its movement back through the

upper compartment from left to right, returning to the pump where it started circulating. This vigorous circulation is essential to the maintenance of a uniform temperature in all parts of the thermostat.

Close to the pump a sensitive thermoregulator is also submerged in the water, which is connected through suitable relays to a battery of immersion heaters that supply the heat lost by the water while traversing the bath. Any one of several makes or designs of thermoregulators and immersion heaters are doubtless adequate for this system of control. While the description of control and of water circulation may sound as though it is complicated, as a matter of fact it is relatively simple, and can be assembled at moderate cost from standard equipment by anyone who is at all ingenious.

Emphasis should be laid upon the necessity of keeping the copper dough-container completely submerged during all such observations in order to insure that the temperature of its contents is maintained constant. If the vessel is too buoyant to stand steadily on the false bottom of the water-bath, lead weights should be attached to hold it down. Also it should not be jarred or disturbed during the progress of dough-fermentation tests, since the dough may be caused to fall if disturbed during the later stages of fermentation.

Technicians and cereal chemists who are concerned with fermenting doughs will discern many applications for such a device. It is useful in following the changes in fermentation rate, and fermentation tolerance as induced by the inclusion of different sugars in varying amounts, diastatic enzymes, flour and dough "improvers," and other treatments which are reflected in fermentation behavior. The doughs may be studied at any state, either direct from the mixing machine or after fermentation of more or less duration. Doughs may be removed from the vessel, "punched," and returned for additional periods which simulate commercial or laboratory dough fermentation in preparation for baking. These are only a few of the numerous applications made of it in recent years.

Summary

A simple and relatively inexpensive device, assembled largely from ordinary laboratory equipment, is described which makes possible the measurement of (*a*) fermentation rate in a yeast-leavened dough, and (*b*) the loss of CO_2 from fermenting doughs. A special copper vessel to hold the dough is desirable, which is heavy, rugged, easy to seal tightly, and a good thermal conductor. While the device here described is designed for manual operation, it is possible to add various automatic features. All operations can be conducted at atmospheric pressure.

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THE EFFECT OF SMALL QUANTITIES OF MALTED OAT FLOUR ON THE KEEPING QUALITY OF WHEAT FLOUR

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(Received for publication May 11, 1939)

The keeping quality of wheat flour has been the subject of many investigations (Swanson, Willard, and Fitz, 1915; Saunders, Nichols, and Cowen, 1921; Whitcomb, Day, and Blish, 1921; and Fisher, Halton, and Carter, 1937); however, it still continues to be a topic of major interest. The fact has been definitely established that a good grade of hard-wheat flour manufactured from wheat that is in good condition will retain its baking quality for a period of time that can be measured in terms of years. In fact there is generally an improvement in baking quality during the first six to ten months of storage. In contrast to the long periods of time during which hard-wheat flour may be stored without deterioration, it is a recognized fact that soft-wheat flours, flours containing self-rising ingredients, and "clear" grade have more limited keeping properties even under good storage conditions.

The question of why the baking value of a clear grade flour will decline as the storage period increases, while the patent flour from which the clear was removed continues to improve with age, is a problem worthy of consideration. As pointed out by Sullivan, Near, and Foley (1936), it is a well known fact that the presence of increasing percentages of wheat germ in flour harms the baking quality in proportion to the quantity present. Clear flours contain a much higher percentage of germ stock than do patent flours. It was generally believed that the fat constituents of the germ were the cause of the harmful effects on baking quality even before there was much experimental evidence to substantiate the belief. It has now been shown by Sullivan, Near, and Foley (1936) that the fat from fresh germ is not deleterious to flour quality and that it is only after unsaturated fatty acids and their subsequent oxidation products

develop during storage that the injurious properties are encountered. Elaborate and complicated theories (Moreau and Dufraisse, 1926) have been built up to explain the effect which certain substances have on the oxidative and hydrolytic reactions of the lipids. Irrespective of the theory involved, the fact is well established that oat flour has antioxidant properties. See papers by Musher (1935) and Conn and Asnis (1937).

The present study was instigated to determine whether additions of malted oat flour to a hard-wheat clear flour could be made to serve effectively the dual purpose of enhancing the diastatic activity of the clear flour and simultaneously reducing the deleterious effects of storage oxidation.

Flour millers have for many years adjusted and standardized the diastatic activity of their flours. This is a necessary control measure because of the unpredictable changes in the diastatic activity of wheat due to causes such as varietal differences, environmental conditions during the growing period, and harvesting and storage conditions. Regulation of the diastatic activity of flour can most satisfactorily be accomplished by adding a small amount of malted wheat flour. The entire subject of the theory and practice in the diastatic treatment of flours has recently been reviewed by Epstein and Schreier (1938).

Description of Experiments

In order to determine experimentally the value of additions of malted oat flour to wheat flour, a strong hard spring wheat clear grade was selected for this investigation. The clear flour was unbleached and undiastated and had the following analysis on a 15% moisture basis: protein 14.7%, ash 0.64%, diastatic activity 187 mg. of maltose. Since freshly milled flour is not normally at the peak of its baking performance the clear was stored in a flour warehouse for three weeks. Following this storage period, the flour was subjected to baking tests as indicated in Table I, test No. 1. The flour behaved in a normal manner during the baking test and showed the usual lack of sustained gas production that is characteristic of undiastated flours, during an extended fermentation period. One portion of this flour was intimately mixed with malted wheat flour and another portion similarly treated with malted oat flour. The two flours thus treated were adjusted to the same approximate diastatic activity (244 mg. maltose) and gassing power as indicated in Figure 1. The diastatic activities were determined by the Blish and Sandstedt (1933) method and the gassing powers were determined by the method of Sandstedt and Blish (1934).

The rates of gas production over a six-hour period for the two flours were remarkably similar. Further evidence that malted oat flour does not differ markedly from malted wheat flour in its action as a diastatic supplement is shown by the baking tests. The bakings conducted immediately following the additions of the two types of malt to the wheat flour were almost identical, as is indicated in Table I, test No. 2. Additional baking tests were conducted at intervals for a period of eight months. Relatively highly diastated flours were purposely prepared in order to exaggerate any differences which the two types of malt supplements might be expected to cause.

Because of the importance of the baking test in this experiment it was not deemed expedient to rely on a single baking procedure. The policy adopted was to use the method of the American Association of Cereal Chemists (1935) with the following variations designed to detect changes in a flour's ability to withstand both extended mixing and fermentation periods.

1. Three-minute mixing time; 3-hour fermentation period.
2. Three-minute mixing time; 4-hour fermentation period.
3. Three-minute mixing time; 5-hour fermentation period.
4. Four-minute mixing time; 3-hour fermentation period.

All bakes were made in duplicate and the bread was scored the following day. Numerical values were assigned to such internal and external characteristics as volume, symmetry, bloom, break, color, grain, and texture. Platt (1931, 1933) has adequately pointed out the difficulties and pitfalls encountered in grading foods, particularly bread; nevertheless when the limitations are understood, scoring continues to be the best criterion of the relative quality of two products. In Table I are recorded the detailed results of the effect of storage, in a warm room, on the baking properties of the two flours under consideration. The data recorded under the heading "Average loaf volume and bread score" in Table I indicate in concise form the changes taking place in the baking value of the two flours, as tested by the four baking procedures, over an extended storage period. There is thus incorporated in the composite values the effect of age on both the mixing and fermentation tolerances of the doughs.

Discussion of Results

The importance of adequate gassing power for the production of quality bread has long been recognized. Baking tests and a record of the gas production over a six-hour period (Fig. 1) indicate that malted oat flour is satisfactory as a diastatic supplement to wheat flour. An inspection of Table I indicates that at the start of the experiment the

unsupplemented clear was improved in baking properties to an almost equal extent by the addition of either wheat or oat malt flour.

During storage both flours consistently declined in baking value. There was a gradual reduction in loaf volume and bread score. The reduction in bread score was the result of decreases in loaf volume, inferior loaf appearance, and less desirable grain. The only grading item which tended to increase the bread score was an improvement in

TABLE I

THE EFFECT OF STORAGE ON THE BAKING PROPERTIES OF A CLEAR FLOUR CONTAINING WHEAT MALT CONTRASTED WITH THE SAME FLOUR CONTAINING OAT MALT SUPPLEMENTS

Test No.	Flour storage period	Type of malt supplement	Baking procedure: 3-hr. fermentation, 4-min. mix		3-hr. fermentation, 3-min. mix		4-hr. fermentation, 3-min. mix		5-hr. fermentation, 3-min. mix		Average loaf volume and bread score	
			Vol.	Score	Vol.	Score	Vol.	Score	Vol.	Score	Vol.	Score
	Days		*cc.*	*%*	*cc.*	*%*	*cc.*	*%*	*cc.*	*%*	*cc.*	*%*
1	21	None	630	91	620	92	520	88	380	76	538	86.7
2	25	Wheat	690	92	680	93	585	89	455	83	603	89.2
		Oat	660	93	680	93	600	90	480	83	605	89.9
3	91	Wheat	685	92	645	90	580	87	440	79	588	87.7
		Oat	610	90	630	90	590	87	470	80	574	86.8
4	122	Wheat	637	92	602	89	475	79	410	75	530	83.7
		Oat	668	92	657	89	515	80	425	74	565	83.7
5	154	Wheat	610	86	625	91	553	86	450	78	558	85.3
		Oat	646	87	692	92	590	85	475	79	602	85.9
6	185	Wheat	560	87	650	93	485	81	445	79	536	85.0
		Oat	590	88	690	94	555	85	450	79	570	86.4
7	248	Wheat	518	85	635	93	518	84	438	76	528	84.5
		Oat	580	85	675	94	582	86	445	76	573	85.3

crumb color. For the first four months there was only a slight difference between the baking character of the two flours and therefore no appreciable evidence that oat flour had made a contribution toward enhancing the keeping quality of the product. However, beyond a four months' storage period it appeared that oat-flour supplements had made a slight contribution toward retarding deterioration. Both the loaf volumes and bread scores of the clear containing malted oat flour had increased, as evident in Table I.

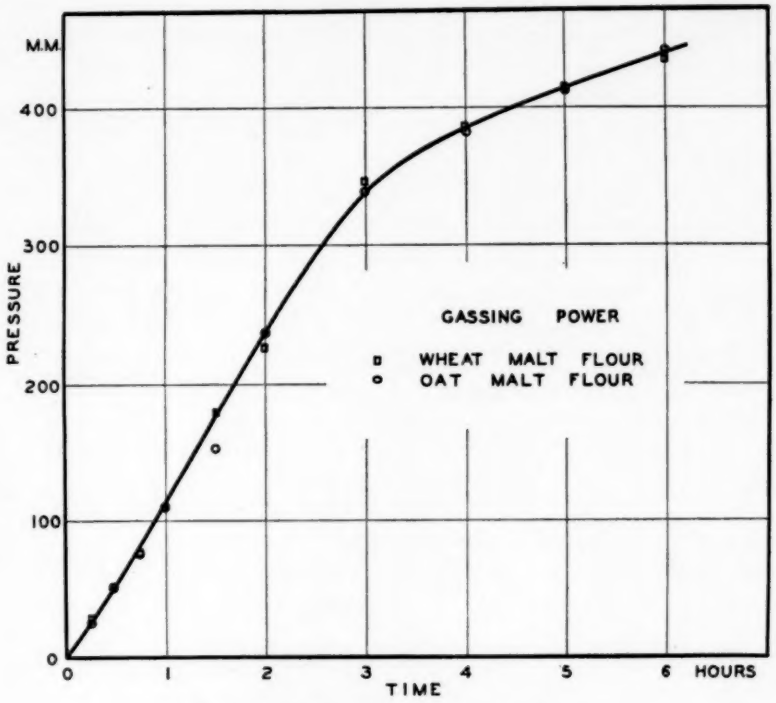

Fig. 1. Gassing power values of the two flours.

Conclusions

Malted oat flour can be successfully utilized to enhance the diastatic activity of wheat flour.

When two portions of a clear-grade flour were adjusted to the same diastatic activity by additions of malted wheat flour to one portion and malted oat flour to the other, it was found that the baking value of the two flours remained practically identical for several months.

Following several months of storage it appears that malted oat flour exerts some influence toward decreasing the rate of deterioration of flour.

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VARIATION IN THE BAKING QUALITY OF WHEAT DURING STORAGE

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(Received for publication May 4, 1939)

At the beginning of any wheat crop movement, urgent demand requires that the cereal chemist immediately determine the baking quality of this new supply. The opinions formulated early in the crop movement almost invariably receive extensive publicity in trade journals and frequently set, for the entire season, the popular conception of the crop's quality. It is important therefore, since wheat is a changing commodity, to consider the state of maturity of the grain and the effect which this may have on the estimation of baking quality.

The effect of storage on the baking quality of wheat has been studied by many investigators. This subject has been adequately reviewed and extensive literature citations have been published in books by Bailey (1925) and Swanson (1938); the results indicate rather conclusively that storage enhances the baking quality of wheat. From these findings it is apparent that freshly harvested wheat, which is necessarily used at the beginning of the harvest period to determine the baking quality of the new crop, is at a low point in its baking-quality

cycle. The validity of the experimental baking test as a means of predicting the true potential baking quality of wheat at this period may be questionable. The present investigation was undertaken to determine to what extent the baking properties of wheat are modified during the first few months after storage and also to ascertain whether the viability of the wheat is related to this change.

Practically all seeds require a certain amount of aging before they will germinate properly. The wheat seed is no exception to this rule. One of the most evident signs of the physical and chemical changes taking place in wheat, after cutting, is the gradual increase in viability. Atkinson and Jahnke (1918) and Swanson (1931) have shown that the modification in new wheat which increases its viability reaches a maximum in the comparatively short time of approximately two months.

The inferior baking quality of dead wheat has been shown by Swanson (1926). Since zero viability rather than appearance is the criterion of the extent of damage to the baking properties of dead wheat, one would expect a relationship between the viability of wheat and its baking performance. However, it should be recognized that low viability caused by old age or damage is probably fundamentally different from the low viability of newly harvested wheat.

Description of Material

Nine samples of wheat of known harvesting date were obtained for this study. The wide geographical area from which the samples were obtained should reduce to a minimum the possibility of dealing with a preponderance of abnormal wheat resulting from the influence of unusual climate, variety, or harvest conditions. The data presented in Table I indicate the origin, harvest date, classification, test weight, and chemical analysis of the samples. It is evident that wide variations of wheat properties are represented in Table I. Some of the samples had to be shipped long distances, and therefore the first milling and baking tests were conducted when the wheat was approximately two weeks old. The investigation of Fitz (1910) indicated that wheat improves in baking quality during the first few days of storage. It is seldom practical to obtain grain immediately after it is threshed; consequently the majority of baking tests of new wheat are conducted on samples which are at least a week old.

The initial baking tests reported in Table III were conducted, it is believed, on wheat of about the usual age of commercial grain at the time when cereal chemists are formulating their opinion of the baking quality of the new crop.

The wheat samples on arrival were placed in burlap sacks and stored in an unheated warehouse. Portions were removed from the sacks for

TABLE I

DESCRIPTION AND CHEMICAL COMPOSITION OF THE WHEAT SAMPLES

Sample No.	Where grown	Harvest date	Class	Moisture	Protein	Ash	Test weight	Germination: Two wks. after harvest	Germination: Six mos. after harvest
				%	%	%	*Lbs.*	%	%
1	Kansas	June 20	HRW	13.1	12.0	1.74	60.3	47	92
2	Ohio	July 8	SRW	13.4	10.3	1.85	60.5	71	98
3	Nebr.	July 6	HRW	10.1	13.4	1.88	60.1	72	83
4	Kansas	July 5	HRW	12.5	12.6	1.76	58.0	51	93
5	S. Dak.	July 22	HRS	13.1	14.5	1.94	57.5	60	97
6	N. Dak.	July 27	HRS	12.0	12.9	1.80	61.1	79	91
7	Wash.	Aug. 4	HW	9.1	14.4	1.89	60.5	97	98
8	Minn.	July 25	HRS	12.0	15.6	2.00	53.0	88	82
9	Mont.	Sept. 19	HRS	9.3	12.1	1.74	59.8	95	98

TABLE II

AVERAGE ANALYSES OF THE NINE FLOURS OBTAINED FROM MILLING THE WHEAT SAMPLES AFTER VARIOUS STORAGE PERIODS

Storage period	Germination	Flour yield	Protein	Ash	Viscosity	Diastatic activity	Gassing power
Days	%	%	%	%	°*McM.*	*mgs.*	*mm.*
15	73	60.9	11.6	0.42	118	129	223
34	83	60.3	11.5	0.42	120	130	216
106	96	61.2	11.5	0.43	115	117	208
180	93	62.2	11.5	0.44	124	121	224

germination, milling, and baking tests, at the time periods indicated in Table II.

Experimental

In Table II is recorded the average germination test of the nine wheat samples and the average change which took place in the analyses of the samples of flour milled from these wheats periodically during a six months' storage period.

The viability of the seeds was tested by making germination determinations. The first germination test, made when the samples were approximately fifteen days old, varied from a low value of 47% germination to a high value of 97%. The average for all samples was 73%. After a month's storage the average germination for all samples had risen to 83%, and the maximum value was reached approximately three months after harvesting; also the best baking results were ob-

tained at this period of maximum viability. This point will be elaborated later.

The wheat samples were milled on the experimental mill described by Libby and Shellenberger (1938). The mill was operated under the temperature and humidity conditions prevailing in a commercial flour mill. The milling procedure paralleled closely that described by Markley (1936). Each of the nine samples was milled four times during the six months' storage period. Every effort was made to reproduce, as closely as possible, the same milling procedure each time the samples were remilled.

The operation of an experimental mill for wheat testing always adds an additional variable. Geddes, Bergsteinnson, and Hadley (1933) have shown that the differences in flour characteristics due to experimental milling are not without their influence on the baking test.

The information recorded in Table II indicates that the ash content of the flour is the only determination that shows a consistent trend. Although it is true that the utilization of the readily convertible carbohydrates of the wheat kernel, by the process of respiration, tends to leave a higher percentage of ash in wheat after prolonged storage, nevertheless no such increase as 0.02% can be accounted for on this basis during a storage period of only six months. The indicated increase in ash content of the flour is probably the result of variations in the operation of the experimental mill as indicated by the flour yield data. The protein, viscosity, diastatic activity, and gassing-power values remained relatively constant.

Because of the importance of the baking test in this experiment, it was not deemed expedient to rely on a single baking procedure. The policy adopted was to use the basic A.A.C.C. procedure (Blish, 1928) plus the following three supplementary methods:

(1) The addition of $\frac{1}{4}$ of 1% malt flour,
(2) The addition of $\frac{1}{4}$ of 1% malt flour plus 1 mg. of potassium bromate,
(3) The addition of $\frac{1}{4}$ of 1% malt flour plus mechanical modification of the dough.

Each sample was baked by all four methods on two successive days, and the average loaf volume and bread score of the two bakes were recorded. Thus the values for loaf volume and bread score recorded in Table III represent the average of 36 test loaves, and each flour sample was baked and scored 144 times during the course of this investigation. Bread scores were determined by grading the bread the day following baking. All the important internal and external characteristics of the loaves were considered during the scoring process.

TABLE III

AVERAGE LOAF VOLUME AND BREAD SCORE OF THE NINE SAMPLES OF WHEAT AS DETERMINED BY FOUR BAKING PROCEDURES DURING A SIX MONTHS STORAGE PERIOD

Storage period	Absorption, 15% m.b.	Baking procedure								Average for all bakes	
		A.A.C.C.		Malt flour supplement		Malt plus 1 mg. of $KBrO_4$		Malt plus extended mixing [1]			
Days	%	*Vol.*	*Score*	*Vol.*	*Score*	*Vol.*	*Score*	*Vol.*	*Score*	*Vol.*	*Score*
15	57.3	485	81.6	551	86.8	636	91.4	600	81.3	567	85.3
34	57.7	496	82.9	583	89.5	649	92.1	597	81.8	581	86.5
106	58.1	460	82.9	576	89.4	638	92.4	576	90.4	564	88.8
180	57.9	458	81.0	566	88.6	634	92.0	601	91.4	568	88.4

[1] Mixing time 4 minutes in a Hobart-Swanson mixer.

Discussion of Results

No conclusions were attempted based on the study of an individual wheat sample, only the average changes which all samples underwent being considered in this report. It is believed that the data and findings herein presented afford a reliable indication of the changes which wheat undergoes during normal storage.

The mean germination for all samples two weeks after cutting was 73%. Two of the wheat samples, because of shipping conditions, arrived with only slightly over 9% moisture; and these two samples had unusually high germination capacity. The fact that the careful drying of grain enhances its germination energy has been recognized and utilized in the malting industry (Kropff, 1927), and therefore the desiccation the wheat samples sustained in this case can be considered a contributing cause for the high average value of 73% germination 15 days after cutting. The increase in viability as the storage period increased indicates that the samples were normal in this respect.

In Table III is recorded the mean absorption, loaf volume, and bread score, as determined by the four baking methods, of all nine samples, for each of the four storage periods. This information is presented graphically in Figure 1. A portion of the area under the loaf-volume curve is sectioned to set it apart from the three upper curves because the ordinates represent different units. A casual inspection of the loaf-volume curve might suggest that a very significant change in the volume had occurred, but actually the mean values for all bakes differed by only 17 cubic centimeters. The average of all baking data indicate that the loaf volumes remained remarkably

constant during the six months' period; however, loaf volume is only one of the many characteristics of bread which should be considered when evaluating baking quality. When all the important internal and external characteristics of the bread are considered it becomes evident that as the wheat matured there was an improvement in the baking quality. Considering the bread score as the criterion of baking quality, it is evident that the best bread was produced when the wheat was approximately three and one-half months old. Also this storage period coincides with the maximum dough absorption and greatest germination capacity. The bread score values, however, indicate

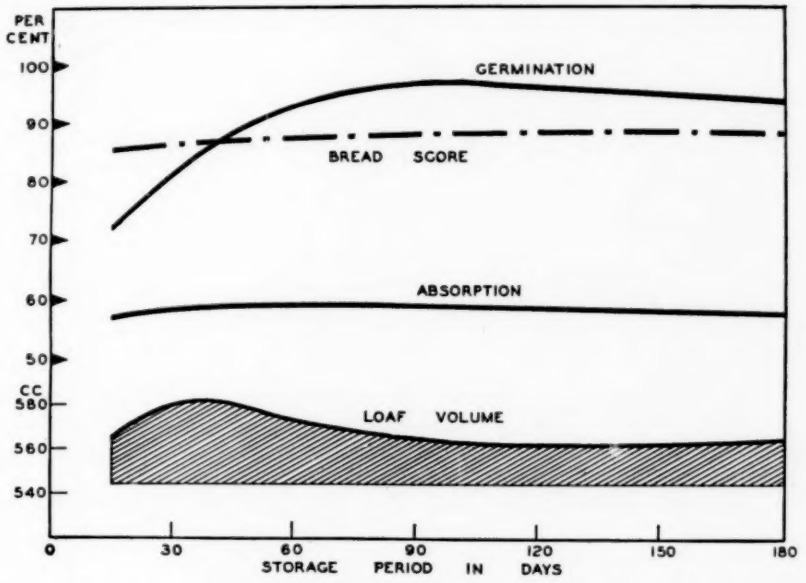

Fig. 1. Effect of storage on germination, absorption, loaf volume, and bread score of wheat samples.

that the improvement noted in baking value is not particularly impressive. In fact, the data indicate that a surprisingly true picture of the potential baking value of wheat can be obtained almost immediately after harvest, assuming, of course, that the grain has been allowed to mature properly before cutting.

Conclusions

The baking quality of wheat is improved by storage after harvest, but the betterment observed during this investigation was not particularly impressive. This conclusion is based on the study of four classes of wheat, obtained from eight states, during the 1938 harvest.

The experimental baking test, applied to new wheat, provides a reliable indication of the potential baking quality of the crop.

There appears to be a direct relationship between the viability of wheat and its baking quality.

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EFFECT OF TEMPERATURE ON DOUGH PROPERTIES, II

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(Read at the Annual Meeting, May 1939)

In the ordinary baking of bread a crust is quickly formed which interferes with the study of the properties of the interior dough during baking. The changes in the properties of dough, while baking, progress in sequence from the exterior to the interior; thus no two zones are under the same condition of change at the same time. In order to obviate these difficulties a method of heating bread electrically in which no crust is formed, and in which the entire mass rises in temperature uniformly and under controlled conditions, was developed by Baker (1939).

Figure 1 is a drawing of an improved baking pan showing the relation of the various parts to each other. Believing that the proper-

ties of the dough determine the character of the bread, we have used various means to test the dough while being heated, so that its changes could be detected and studied. The rate of rise becomes a measure of volume because the pan is straight-sided and thus restrains the dough between the electrode walls throughout its oven spring. In order to obtain a uniform heat input in the dough, a constant wattage is applied. The voltage required to keep this wattage constant varies with the changes in resistance of the dough and therefore is inversely indicative of the changes in conductivity of the dough while baking. The passage of a weight through the dough mass while heating gives evidence of

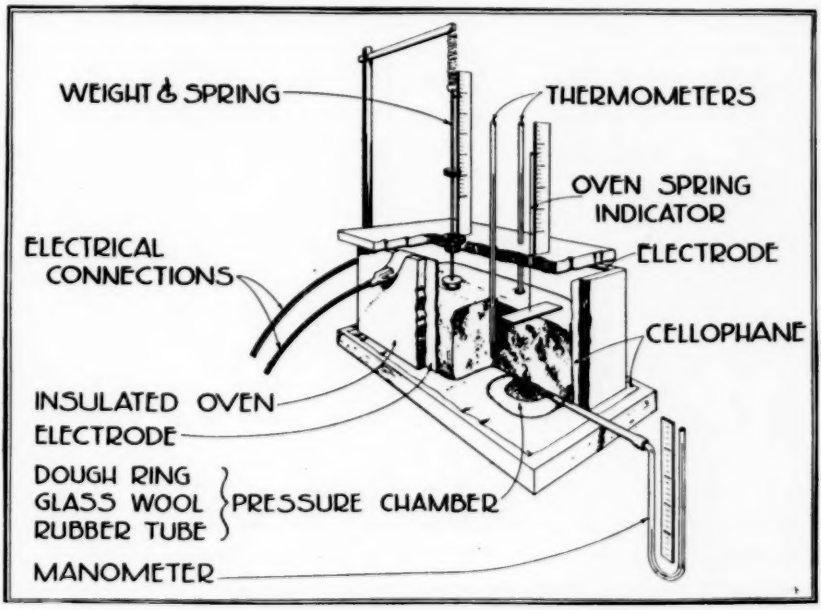

Fig. 1. An improved baking pan.

the changes in plastic properties of the dough. Lastly, a chamber was devised underneath the dough, communicating to a manometer which records changes in pressure within the dough structure itself, whereby the forces operating within the dough causing its expansion are indicated.

Numerous difficulties developed in the apparatus previously described. By changing the size of the pan to 9 inches long, $3\frac{3}{4}$ inches wide, and 6 inches high, and reducing the current to 150 watts, more uniform heating was obtained. Electrode surface phenomena appeared to be causing unequal resistance and unequal distribution of heat and interfered with the conductivity-voltage relationship. This

difficulty was corrected by coating the electrodes with an alcoholic solution of quinhydrone which, acting as a chemical reservoir, would store up the reactions of the alternating current so that no local formation of gases occurred and perfect adherence of the dough mass to the electrode was obtained. This coating has given very satisfactory performance, as indicated by the reproducibility of results.

Numerous metal parts used in the earlier apparatus tended to short-circuit the current and have been entirely eliminated by making the entire chamber, except the electrodes and the falling weight, of non-metallic substances which are good insulators. For this reason the use of metallic thermometers was abandoned and glass mercury thermometers adopted, with increased accuracy as to reproducibility and with less lag in following the actual temperature of the dough. The falling weight was redesigned so that the portion penetrating the dough would present the minimum surface to the dough. It still has sufficient cross-section to make a tunnel through the dough which does not close above the weight and adhere to the shaft. Additional weight is carried on the shaft above the plunger to force it through all types of dough. This necessitates the supporting of the plunger by means of a spring, which partially supports the weight at the moment of penetration and totally supports it when the weight reaches the exact bottom of the pan. The weight of the plunger used when entering the dough is 46 g. Its diameter is 15 mm., with a tapered point at an angle of 60°. This spring also assures a vertical movement of the plunger.

The motion of the plunger is complicated not only by the effect of the spring upon the weight but by the upward motion of the dough during baking. It is necessary to correct the motion of the plunger in accordance with the motion of the dough and in proportion to the position of the plunger in the dough at each reading. The movement of the plunger through the dough and the upward movement of the dough (oven spring) are plotted in the same units so that their motion relative to each other can be seen.

To measure the pressure at the bottom of the chamber, difficulties were encountered in the earlier device because the bond between the dough and the bottom of the pan was often not sufficient to retain the pressure, and gases escaped. This difficulty has been obviated by the use of the heaviest non-waterproof cellulose cellophane obtainable. This cellophane has the property of adhering to dough with greater strength than the bonds within the dough substance itself. To further assure that the gases will not be lost from the chamber by passage underneath the dough, the chamber is surrounded by a ring of unleavened, unsalted dough. Such a ring of dough presents an additional

barrier to the passage of gas out of the chamber by any path other than through the test dough itself.

The skin which forms over the outside of a dough during proofing is a barrier which is penetrated by the plunger with difficulty. Because of the increased strength of the skin the pressure required inside of the loaf to cause expansion is increased. In order that the values measured be indicative of the true properties of expanding dough, the top of the loaf is slit just prior to inserting the instruments, thus eliminating this undesirable effect of the exterior skin.

In the work of Baker and Mize (1939) the following changes are reported to occur as the temperature of the dough is elevated:

(*1*) Increase in volume of the gases within the dough is caused by thermal expansion, by gas being driven out of solution and by the increased rate of formation of gas by the yeast. This expansion of gas in the dough produces pressure and thereby causes elastic and plastic extension of the dough, as evidenced by oven spring.

(*2*) A softening of the dough occurs during heating, as shown by the change in the rate at which a weight drops through the dough.

(*3*) This softening process is quickly arrested by the starch swelling at 136° F. The swelling of the starch produces the following changes in the properties of the dough because of the water removed from the other dough ingredients by the swelling starch: *First*, the starch granules by their increased volume become fixed in location and move about in the dough mass with difficulty as further stretching goes on. *Second*, water is taken from the gluten by the starch. The gluten properties are thereby strengthened, becoming more viscous and more elastic because of this dehydration. *Third*, the transfer of water in the system increases the electrical resistance and is accompanied always by a rise in voltage.

(*4*) The swelling of the starch is accompanied by a slight decrease in rate of temperature rise, indicating an increased absorption of energy. This reaction is largely completed within a very short period of temperature rise, though further swelling of the starch may occur during the continued heating of the dough.

(*5*) Destruction of yeast seems to begin during the same temperature range as starch swelling, but is not completed until the temperature is much higher.

(*6*) In desirable doughs, oven spring continues during and after primary starch swelling and is characterized by a rising pressure within the dough. The expansion of doughs, not previously arrested, ceases during gluten coagulation. At the usual rate of heating during baking, gluten coagulation does not perceptibly begin until after 165° F. has

been reached, and progresses slowly. Long-continued heating is necessary for complete coagulation of the gluten.

(*7*) Alcohol and water are distilled from dough, thereby furnishing an additional volume of gas which may produce pressure and which, by heat of evaporation, holds down the temperature during their distillation. These gases upon escaping from the bread sweep out carbon dioxide.

(*8*) There may be a marked increase in electrical resistance in many doughs toward the end of baking, particularly those which have been over-oxidized.

Experimental

Oven spring.—Dough expansion during heating is determined by two properties in the dough: first, by the increase in volume of gas, and secondly, by the amount of gas which is retained.

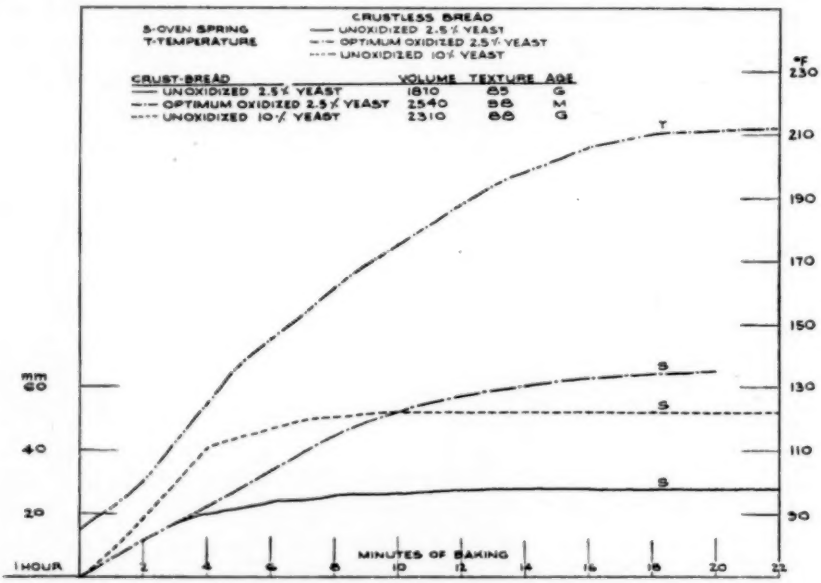

Fig. 2. Relation of oven spring to amounts of yeast and oxidation.

Figure 2 shows the oven spring of, first, an unoxidized, no-time dough; second, a similar dough modified by optimum amount of oxidation; and third, another similar dough modified by using four times as much yeast. The doughs containing different amounts of yeast increase in volume from the beginning of heating at markedly different rates. With the same amount of yeast, the unoxidized dough expands at the same rate as the oxidized dough until it reaches a point at which gas is not held in sufficient quantity to maintain that

rate of expansion. It is to be noted that the oxidized dough continues its oven spring during a long period of the heating cycle until the zone of gluten coagulation has been entered. To obtain this oven spring, it is necessary for the dough mass to continue its stretching and plastic flow after starch swelling has produced its profound changes upon the dough properties. It is such flow that enables a loaf to produce a smooth, shredded crust during oven spring.

The effect which any change in dough composition or handling will have upon oven spring depends upon whether the change alters gas production or gas retention. Those changes which affect gas production give oven spring differences which appear at once upon heating. Those changes which increase gas retention give oven-spring characteristics that appear during a later portion of the heating period.

The above doughs and all succeeding doughs, unless otherwise noted, are from the same Kansas patent flour baked as no-time doughs. The doughs were panned immediately after mixing and in all cases baked when proof height was reached. No-time doughs and patent flour were used for these experiments to show as far as possible the effects of each variable upon the dough without modification by fermentation and enzymatic action. This use of no-time doughs magnifies and makes distinctive their differences and simplifies the interpretation of results.

Before proceeding to the next chart it will be desirable to review briefly the work of Halton and Scott Blair (1937). Broadly these investigators and co-workers have found that dough quality is determined by the relation of viscosity to the modulus of elasticity. The viscosity should be as high as possible and should not fall too rapidly with addition of water or with fermentation or with increasing stress. They also state that a suitable modulus of elasticity is necessary. One can readily see that unless a dough is highly viscous the elastic properties do not come into play. If you increase the viscosity, the dough will stretch elastically. If the viscosity is too low, it will not stretch but will flow and run. A dough may have a fine elastic gluten but if its viscosity is low and the dough runny, it will not stretch. A green dough is of that type.

Plunger-weight (W) and pressure (P) measurements.—During the baking of the same three doughs shown in Figure 2 we measured the fall of a weight through the dough and the changes in pressure occurring while baking. In Figure 3 these measurements are superimposed upon those shown in the previous figure. It is to be noted that with the larger amount of yeast, substantially no difference occurred in the fall of the weight through the dough nor the pressure produced in the dough by the expanding gas. On the contrary, the same dough when

oxidized and baked with the smaller amount of yeast permitted the weight to drop more slowly and gave marked pressure characteristics. The pressure rose substantially throughout the entire course of the baking.

Reasoning from Scott Blair's ratio it is apparent that oxidation has increased the viscosity of this dough, whereas the increased yeast content has substantially no effect on this property in these no-time doughs.

It is particularly to be noted that the oxidized dough supported the falling weight so that there was substantially no motion through the dough until the softening produced by heat weakened its properties

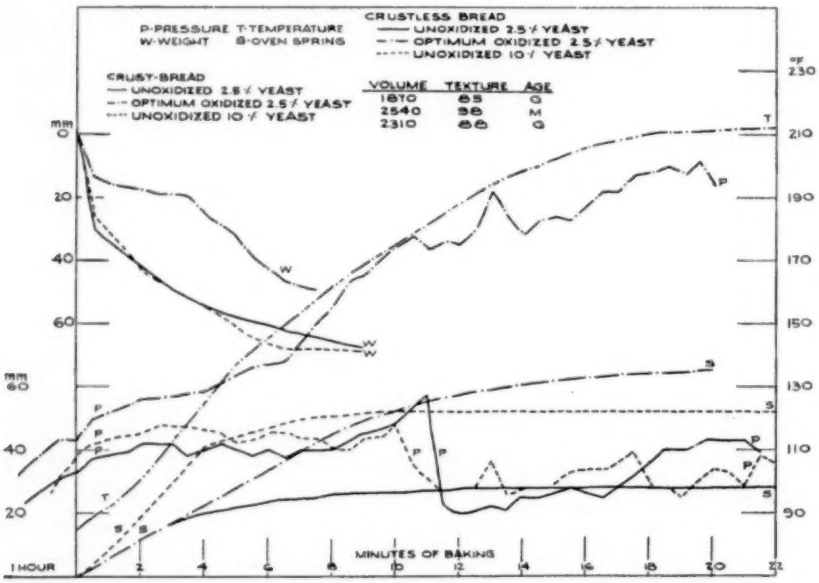

Fig. 3. Relation of oven spring, pressure, and weight to amounts of yeast and oxidation.

and the weight resumed its course downward. This period of arrested motion which because of oven spring is accompanied by an actual upward motion of the weight and thereby increasing force applied to the dough, suggests that the viscosity is so high in this dough that the weight is supported by the elastic properties of the dough and held there until the softening by heat permits the dough to flow and release the weight.

The movement of the weight shown here with the oxidized dough will usually be found characteristic of good doughs whether obtained by oxidation or by other means. The changes in dough which produce undesirable results are those associated with excessive softening and

loss of viscosity during heating. All doughs soften but those which produce desirable results are associated with a lesser amount of softening, indicating that they retain sufficient viscosity and elastic extension to carry the dough structure to the point where the swelling of starch by removal of water increases its strength.

Doughs which make good bread have a high initial pressure and exhibit a rising pressure while baking. If the pressure during the earlier stages of baking does not rise, poor bread is obtained. If the pressure does rise then good texture is retained in proportion to the duration of the rise and to the slope of the pressure curve. The steeper the slope and the longer its duration, the finer the texture found in the resulting bread. However, later graphs will show that on any type of pressure curve, should the pressure fall prior to or during starch swelling, then oven spring ceases and small volume is obtained. But good texture is retained if the pressure starts from a high level and the slope is an ascending one prior to the fall.

Voltage measurements.—Table I shows the influence of proof volume of a dough upon the voltage required to momentarily pass 150

TABLE I

VOLTAGE REQUIRED TO FORCE 150 WATTS OF 60-CYCLE CURRENT THROUGH 540 GRAMS OF DOUGH AT VARIOUS PROOF HEIGHTS

Time	Temperature	Proof height	Voltage
Min	°*F.*	*mm.*	
0	86	36	73
10	86	45	75
20	87	53	77½
30	87	62	80
40	88	73	82
50	88	82	85
60	89	92	87
70	89	100	88½
80	89	107	90½
90	89	112	91
100	89	112	92
110	89	112	91½
120	89	113	92
130	89	112	91½
140	90	108	89½

Note: Regular proof height is 90 mm.

watts of electricity through the dough. It is to be noted that the resistance of the dough progressively increases as the proof height increases. Inasmuch as the electrode surface utilized is constantly increased during proof, the increase in voltage can only be due to changes within the interior of the dough which increase the electrical

resistance. This is believed to be due to the increasing diameter of the expanding bubbles, which thereby causes the electrical path to become longer.

Voltage curves indicate the relative conductivity of doughs during the heating and show how the conductivity is affected by water, salt, dough composition, volume of the dough, and hydration of the dough ingredients at the starch swelling point and also toward the end of the heating period.

The suggestion in paper No. I of this series that a voltage rise in these curves was due to differences in gluten coagulation brought about by oxidation is not supported by further investigation. Following the method of Alsberg and Griffing (1927) no difference in alcohol solubility was found between oxidized and unoxidized cooked doughs, although these gave a wide divergence of voltage near the end of the heating period. Neither was there a difference found in degree of dispersion with sodium salicylate when used in a method suggested by Rich (1936) and modified for doughs by Dr. H. K. Parker, to whom we are indebted for these observations. This increase in resistance, however, must be associated with the transfer of water in the system and therefore suggests that oxidation has increased the hydration capacity of the dough.

The following charts show how dough properties are changed by varying the composition of the dough.

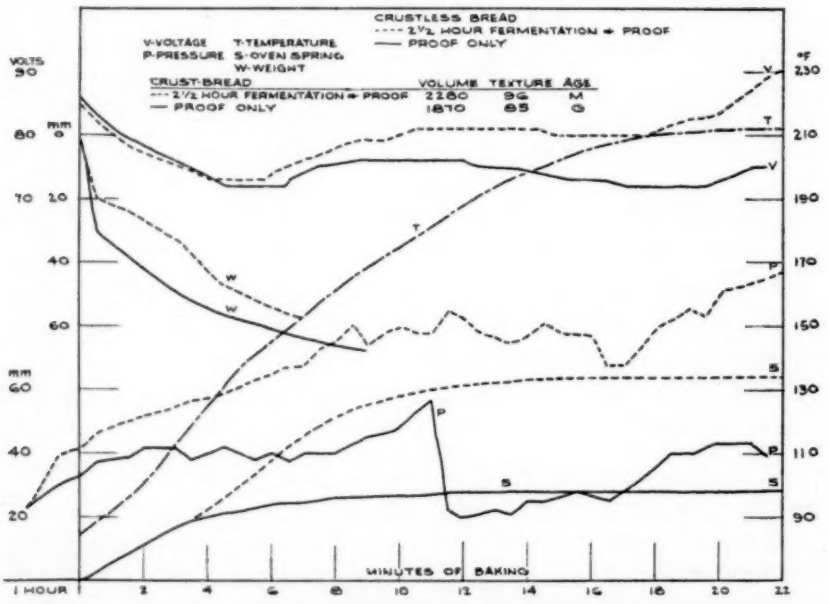

Fig. 4. Effects of fermentation on unoxidized dough.

Effects of fermentation.—Figure 4 shows the effects of fermenting a dough to which sufficient sugar was added. After two and a half hours of fermentation the dough was placed in the electric pan and handled as before. The fermentation has apparently changed the properties of the dough, as is shown by the graph. It has changed from a dough which had low viscosity and did not retain the pressure, to one that holds the pressure well. The rise in pressure is smooth until oven spring is almost completed, when it becomes irregular. The gas is now coming out of the dough in puffs. It seals up again, puffs out again, seals up again, and so on. The property of resealing after gas escape is a very important thing in dough.

The fall of the weight through the dough is changed materially by fermentation and shows a viscosity increase. The weight slows up quickly but heating again softens the dough and the weight movement is again increased and then slows as the starch swells. Apparently the effects of fermentation and oxidation are somewhat similar.

It is to be noted that the divergence in voltage which occurs is substantially paralleled by the divergence in volume of the two loaves except toward the end of the heating period.

Effects of water.—The ingredients in the doughs for Figure 5 were the same as the optimum oxidized dough in Figure 3, except for absorption. In Figure 3, 66% absorption was used; in the stiff dough in Figure 5, 61% water was added and the thin dough had 71% water therein. The stiff dough gave better texture and less volume. Both made good bread and gave a high initial pressure and steady pressure rise during baking. The steeper ascent of the pressure in the stiff dough indicates a more favorable viscosity-elasticity ratio and should give the better texture of the two. The movement of the weight also shows the stiff dough to be very viscous, so that the weight was supported for a long time at a high level and the softening by heat delayed. The thin dough had its viscosity and elasticity both lowered so much that the weight was not supported at any time. However its ratio is more favorable, as shown by the slower motion and distance traveled, than in the untreated doughs of lesser water shown previously in Figure 3.

The difference in conductivity is due chiefly to the difference in water content. The final voltage rise is probably due to the taking up of water by the dough ingredients. In the stiff dough this rise is higher because of its much lower content of free water.

Effects of shortening compounds.—Figure 6 shows three doughs, one baked with commercial hydrogenated shortening, one with refined cotton-seed oil, and the third with no shortening. These doughs were oxidized to make good bread and were identical in all respects except

for the change in shortening noted. The dough containing hydrogenated shortening gave a large, fine-textured loaf of commercial crust bread. The doughs with fluid shortening and no shortening gave small, very fine textured loaves of bread. The only outstanding diffrences among the three loaves were in volume. Similar volume and texture characteristics were obtained in the crustless test loaves as shown in the graph. All three loaves gave the same oven spring until divergence occurred, showing that the difference in volume is due to gas retention.

The pressure curves show that the loaf with the standard hydro-

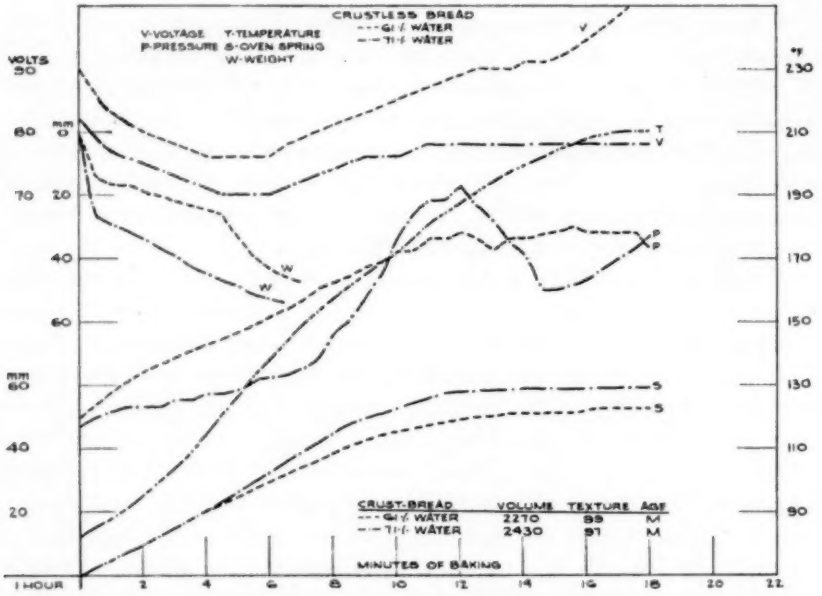

Fig. 5. Effects of water content on optimum oxidized dough.

genated shortening had the usual ascending pressure characteristic of good bread, whereas the two loaves which had the small volume followed also for a period the same ascending pressure, and then a marked drop in pressure occurred. At the same time the oven spring ceased. This drop in pressure continued downward until the swelling of the starch reinforced the properties of the dough and it resumed its ability to retain gas. Then the pressure built up rapidly and steadily to a very high level, with little increase in volume and continued through to the end of the baking period.

The problem here is, Why did these two loaves fail to hold pressure and gas during the critical softening period of the baking, whereas the loaf containing semi-solid shortening gave an entirely different per-

formance? The motion of the weight gives no satisfactory explanation. In all three doughs the falling weight performed in substantially the same manner, giving all the characteristics that would be expected of a dough of fine quality and showing that the elasticity-viscosity ratio of these doughs must be alike in all three cases.

This leads to the conclusion that the difference in these doughs is due to the character of the fats. Tests with other fats indicate that neutral liquid fats act like the cottonseed oil and that the action of semi-solid fats is similar to that of commercial hydrogenated shortening. One is led to speculate as to why this should be. The fine texture of all of these loaves shows that there is no coalescence, nor was the

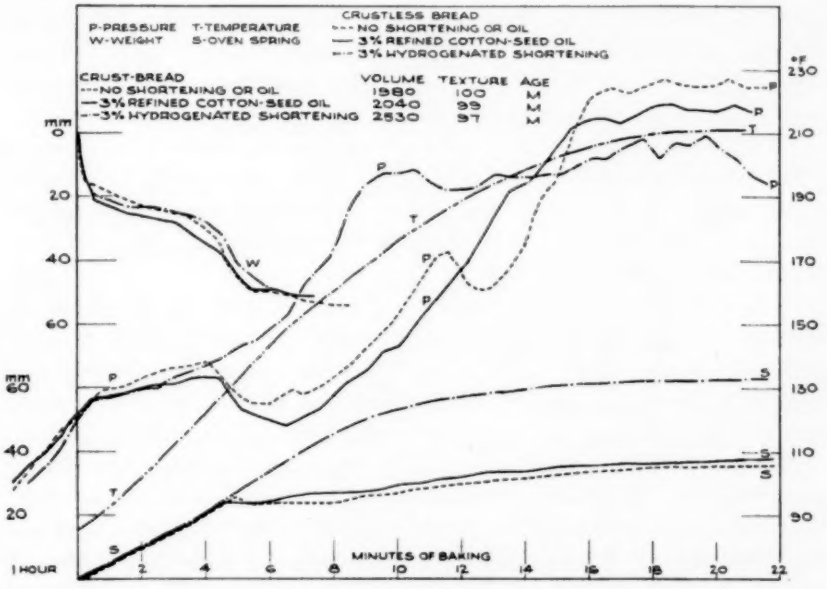

Fig. 6. Effects of shortening on optimum oxidized dough.

cell structure disrupted as was shown by their final ability to retain gas. Apparently gas must escape from these doughs during the softening period by diffusion through the cell walls; hence the difference in their ability to retain gas may be a difference of cell-wall porosity. Here is a clew: solid shortening may prevent cell-wall porosity, whereas fluid shortening or no shortening is unable to do so. Possibly the solid shortening is present throughout the proofing and early baking period as originally distributed in the mixture, whereas the fluid shortening disperses further during the proof. As the critical point in the dough is reached the semi-solid shortening melts and closes the pores so that gas escapes with more difficulty; hence the dough is

enabled to continue its expansion until starch swelling reinforces the entire structure. The liquid fat, being dispersed before baking, is unable to produce this effect.

Summary

A method of testing dough described by Baker (1939) which gave means for indicating the electrical conductivity, the plasticity, the oven spring, the temperature, and the pressure required to extend the dough while being heated from 85° to 212° F. has been further investigated.

The apparatus has been improved, so as to prevent heat loss or gain, to produce more uniform heating of the dough, to assure a more constant conductivity-voltage relation, to measure more accurately the plasticity of the dough by means of a falling weight, and to indicate more accurately the pressure within the dough by the manometer attached to the redesigned gas chamber.

By the use of this redesigned apparatus it was found that changes which affect gas production in a dough give oven-spring differences which appear at once upon heating, while changes which increase gas retention give oven-spring characteristics that appear during a later portion of the heating period.

The voltage measured during baking is affected by the temperature, absorption, salt content, dough composition, volume of dough and hydration of the dough ingredients. However, where the compositions of doughs are similar except for fermentation or oxidation, differences in voltages are caused largely by changes in volume and hydration.

The motion of a weight through dough is controlled by its plastic properties and is greatly influenced by relationship of viscosity to elasticity. Oxidation, fermentation, and low absorption may decrease the movement of the weight through a dough and alter the properties sufficiently almost to arrest its motion. All doughs soften under the influence of heat, so that the motion, if arrested, is resumed and continues down until arrested by swelling of the starch.

The pressure measured, as here described, indicates the cell pressure in the dough. Desirable doughs develop high pressure in the proof; during baking the pressure rises continuously into the gluten coagulation period. The more rapid the rise in pressure, the better the bread obtained. Any interruption in the pressure rise is accompanied either by loss in volume or poor texture. The rapidity at which the pressure rises is controlled by the Scott Blair viscosity-modulus ratio. The steeper the slope, the higher is the viscosity.

During the baking of well oxidized, no-time doughs, made without shortening, a softening occurs as shown by the falling weight. This

is accompanied by a drop in pressure and the stopping of oven spring. Liquid shortenings used to the extent of 3% show no substantial alteration of these effects. On the contrary, doughs made with 3% semi-solid shortening, when the period of softening occurs, show no fall in pressure and no slowing of oven spring. This difference in behavior indicates that doughs containing no shortening or containing liquid fats may become porous, allowing the expanding gas to escape during the softening period, while doughs containing semi-solid shortening are able to retain much of the gas until after starch swelling and on into the zone of gluten coagulation.

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THE PROTEINASE IN WHEAT FLOUR[1]

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(Read at the Annual Meeting, May 1939)

Evidence of the occurrence of a proteinase in flour has been cited in a previous paper (Balls and Hale, 1936). Some of this evidence goes back to 1884 (Balland) and more has since accumulated. Contributing thereto were the extraction and purification of a proteinase from bran and whole wheat, and its recognition as an enzyme of the papain type, as reported from this laboratory (Balls and Hale, 1935 to 1938). The proteinase of sprouted wheat may prove to be the same or a very similar enzyme, for Mounfield (1938) in an investigation of its properties has observed that its action on edestin (though not on gluten) is accelerated by cyanide.

The conclusion that the proteinase in flour is also a papainase, like that obtained from whole wheat, follows logically from the behavior of paste, dough, and gluten toward oxidizing and reducing agents known to influence the activity of both papain and the wheat

[1] Food Research Division Contribution No. 432.

proteinase. Jørgensen has arrived independently and contemporaneously at this conclusion regarding the papain-like nature of the flour enzyme (1935, 1936, 1939). His work has definitely established the point at issue and the present paper aims only to furnish the type of direct evidence therefor that is based on the actual extraction of the proteinase from patent flour and the observation of its behavior in solution toward well-known activators and inhibitors of papain.

Like papain and the proteinase from bran, the enzyme isolated from patent flour is activated by sulphydryl compounds and inhibited by ascorbic acid, iodoacetic acid, cystine, and various oxidants of the bread-improver type. There seems to be no reason for changing the opinion expressed by Balls and Hale (1935, 1936, and 1938) that the action of air, bleaching agents, and bread improvers in modifying the baking properties of flour depends on a more or less complete inactivation of the proteinase.

Extraction of the Enzyme from Flour

One kilo of unbleached patent flour was extracted for 24 hours at 0° with 4 liters of 10% sodium chloride solution containing a trace of cysteine. After being centrifuged in the cold the supernatant liquid, with still another trace of cysteine, was made 0.4 saturated with ammonium sulphate and left for 24 hours longer at 0°. The precipitate was then centrifuged out and discarded. The supernatant liquid was next made 0.8 saturated with ammonium sulphate and allowed to stand for an additional 24 hours at 0°. The precipitate was then filtered off and dried on a porous plate. The yield was 30 grams of dried material.

Ten grams of the dried preparation were dissolved in 60 cc. of a 15% glycerin solution containing a trace of cysteine, and dialyzed under pressure overnight against 15% glycerine solution at 0°. The inactive protein that precipitated during the dialysis was then centrifuged out. The resulting solution contained 1.06 mg. of protein nitrogen per cc. It represented, therefore, about a tenfold increase in purity over the original flour, but none over the original flour proteins. Such a preparation, however, possesses the great advantage that it can be used in a homogeneous system, thus excluding questions of extraction, diffusion, and adsorption on accompanying solids.

Method of Estimation

The dialyzed solution served as a reference with which to compare the activities of other preparations of the enzyme. A set of empirical curves similar to those used for the estimation of the enzyme from

wheat bran was made, using various quantities of the dialyzed solution. Figure 1 shows the curves obtained, expressed in terms of the loss of viscosity per milligram of protein nitrogen in the enzyme preparation. Measurements were made after four different times of digestion.

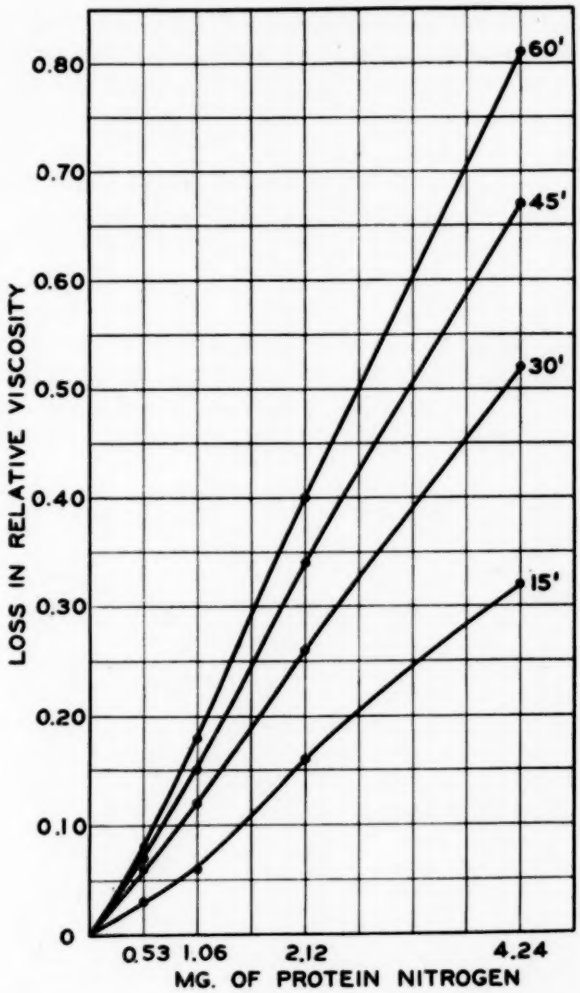

Fig. 1. Loss of viscosity per milligram of protein nitrogen in the enzyme preparation.

The apparatus, solutions, and method used for determining the change in the viscosity of gelatin are the same as those described in the previous paper (Balls and Hale, 1938), with the exception that the activity values are here expressed as milligrams of protein nitrogen in that quantity of the reference preparation showing the same activity.

Properties of the Extracted Enzyme

The dialyzed preparation just described was used to determine the effect of several oxidizing and reducing agents on the activity of the enzyme. Activation by cysteine or cyanide and inactivation by iodoacetic acid and oxidizing agents have been observed to differentiate papain from the other types of proteolytic enzymes. It is not necessary to discuss the reasons for this behavior of papain when the question is merely one of identifying the enzyme. It is commonly held that the presence of a sulphydryl group in the enzyme protein is essential to its activity.

The flour proteinase is activated by cysteine and inhibited by cystine, bromate, persulphate, metavanadate, and iodoacetic acid. Ascorbic acid was also found to be a powerful inhibiting agent, thus corroborating Jørgensen's results on whole flour (Jørgensen, 1935a). The data are given in Table I. No fundamental difference in behavior toward activating and inhibiting agents has been observed between the enzyme prepared from flour and that from bran or whole wheat. The isolated flour enzyme appears likewise to be a papainase.

TABLE I

ACTIVATION AND INHIBITION OF THE PROTEINASE FROM FLOUR

Activity values refer to mg. protein N in the amount of reference preparation showing the same activity.

Volume of enzyme solution *cc.*	Activity after treatment for 30 minutes					
	No additions	Cysteine	$KBrO_3$		$K_2S_2O_8$	
		10 mg.	1 mg.	2 mg.	1 mg.	2 mg.
1	0.8	1.1	—	—	—	—
2	2.0	2.3	0.5	0.2	0.3	0.3

Volume of enzyme solution, *cc.*	$NaVO_3$		Iodoacetic acid	Ascorbic acid	Cystine	
	1 mg.	2 mg.	M/100[1]	1 mg.	2 mg.	10 mg.
2	0.8	0.3	0.2	0.4	0.7	1.3

[1] Concentration in the enzyme solution.

A curious property of the enzyme from either source is that it is made irreversibly inactive by dilution with water, while the addition of cysteine before dilution seems to protect the enzyme, at least to some extent, from this decomposition. Table II summarizes the results from three experiments showing this behavior.

The proteolytic activity of the flour enzyme has also been observed with casein as shown in Table III. The method of determining casein digestion was that of titration in alcohol after incubation of the protein and enzyme at pH 5.0, as is frequently done in the assay of papain (Balls, Swenson, and Stuart, 1935). While considerable splitting of casein occurred in two hours, very much larger values were obtained in 20 hours. As with the preparations from bran, this is an indication, although no definite proof, that a peptidase may accompany the proteinase.

TABLE II

INHIBITION OF THE CRUDE FLOUR PROTEINASE BY DILUTION WITH WATER AND PROTECTION THROUGH THE PRESENCE OF CYSTEINE

Treatment	Activity—mg. protein N in corresponding amount of reference preparation
1 g. prep. B (from bran) dissolved in 39 cc. water; thereafter 100 mg. cysteine added. Assay on 4 cc. after standing 30 min.	0.0
1 g. prep. B mixed with 3 cc. water containing 100 mg. cysteine. After standing 30 min. diluted to 40 cc. with water. Assay at once on 4 cc. of the dilution.	1.1
1 g. prep. C (from bran) dissolved in 49 cc. water; then 100 mg. cysteine added. After standing for 30 min. 4 cc. was analyzed.	0.0
1 g. prep. C mixed with 2 cc. H_2O containing 100 mg. cysteine and allowed to stand 30 min. It was then diluted to 50 cc. and 4 cc. analyzed at once.	1.6
500 mg. prep. 2 (from flour) dissolved in 10 cc. water; 1 cc. of this + 10 mg. cysteine stood for 30 min.	0.0
500 mg. prep. 2 dissolved in 5 cc. water; 0.5 cc. of this + 10 mg. cysteine stood for 30 min.	1.0

TABLE III

CASEIN DIGESTION AT 35° C. BY CYSTEINE-ACTIVATED FLOUR PROTEINASE

Amt. enzyme	Substrate	N/20 KOH after 2 hrs.	N/20 KOH after 20 hrs.
cc.		*cc.*	*cc.*
None	Casein alone	0.00	0.00
2	No casein	0.00	0.00
2	6% casein + 10 mg. cysteine	0.85	3.00
5	6% casein + 10 mg. cysteine	1.25	5.05

The Amount of Proteinase in Flour

If a small quantity of flour made into a thin paste with a cysteine solution is added to gelatin, a significant loss in viscosity takes place. The results of such an experiment are shown in Table IV. They probably indicate little more than a lower limit to the amount of

proteinase present. While the flour proteins were evidently dispersed by the cysteine, they probably reprecipitated when mixed with the gelatin and buffer. This would explain why increasing the fineness of the flour by pulverizing it in a ball mill had no marked effect on the results. When gluten alone instead of whole flour was used, the reprecipitation of the dispersed protein was easily noticed. It usually clogged up the viscosimeter. Furthermore the flour proteins probably compete so successfully for the enzyme with the gelatin that the digestion of the latter is greatly hindered. Thus, when a small amount of crystalline papain was also added to the flour the rate of gelatin liquefaction was not increased, although the quantity of papain was sufficient to digest the gelatin in the absence of the flour. This does not necessarily imply that the papain was inactive, but rather that its activity was here confined almost exclusively to the flour proteins.

TABLE IV

APPROXIMATE AMOUNT OF PROTEINASE IN UNBLEACHED PATENT FLOUR

Sample	Activity—mg. protein N of reference preparation	
	After min.	
50 mg. flour, untreated, mixed directly in the viscosimeter with gelatin containing 10 mg. cysteine. Total vol. 10 cc.	15	1.0
	30	0.8
	45	0.8
	60	0.8
50 mg. flour treated with 10 mg. of neutralized cysteine HCl in a volume of 1 cc. for 30 min. The mixture then added to the gelatin in the viscosimeter as usual.	15	1.1
	30	0.9
	45	1.0
	60	0.9
50 mg. flour, pulverized in ball mill, treated with 10 mg. neutralized cysteine HCl in a volume of 1 cc. for 30 min., then assayed as usual.	15	0.8
	30	0.7
	45	0.7
50 mg. flour, plus 0.001 mg. active crystalline papain, treated with 10 mg. neutralized cysteine HCl for 30 min., then placed in viscosimeter.	15	1.0
	30	0.7
0.001 mg. active crystalline papain + 10 mg. neutralized cysteine HCl tested directly after mixing.	15	1.5
	30	1.2

The data in Table IV indicate, therefore, only the least amount of proteinase that may be present. Nevertheless the flour used reduced the viscosity of gelatin at about the same rate as one fifty-thousandth of its weight of crystalline papain. This quantity of enzyme seems in fact surprisingly large, when one considers the effect of a trace of papain added to dough. One part of commercial papain to twenty

thousand parts of flour may completely liquefy a dough, and a quarter of this quantity of the crystalline enzyme should also suffice. If, as seems reasonable, this marked change is caused by scarcely doubling the proteinase, it follows that the amount naturally present is of no slight importance. There is without doubt enough to produce disastrous effects if by mischance the enzyme should be activated—a situation that can conceivably arise in several ways, for example through the autolysis of dead yeast cells and the liberation of their glutathione. The disintegration of gluten by incubation with cysteine has been previously described (Balls and Hale, 1936a). It was not possible to demonstrate that any proteolytic action had taken place during this treatment; yet proteolysis was by no means ruled out. The gluten undergoes a remarkable decrease in viscosity by the addition of cysteine and although it can be re-coagulated with salts, the resulting gluten is thereafter of very poor quality. The evidence now seems to point to the conclusion that the proteinase is involved in this dispersion of gluten, although the action definitely stops short of an extensive hydrolysis. The data corroborate precisely Jørgensen's statement (1936) that "flour contains a powerful but latent proteinase."

The amount of proteinase in flour is sufficiently striking to justify considerable speculation as to its effect. It is obvious that no thorough-going breakdown of the protein occurs in normal dough; therefore it is reasonable to look for the effects of the flour proteinase in the direction of protein modification, rather than extended hydrolysis. To a greater or less degree all proteinases appear to have the property of producing a clot with various proteins. The clotting of milk by chymotrypsin and of blood by papain are well-known examples. The formation of gluten also has the appearance of being an enzymic clotting. The experimental proof of such a hypothesis would be quite a difficult matter, particularly since it cannot be claimed that proteolysis of flour protein is the only factor involved. Contact between the protein particles is also necessary and some pressure must be applied to cause them to coalesce. Hence the gluten mass is not formed in thin pastes but may be prepared therefrom by centrifuging. However, such behavior in a heterogeneous system like dough does not preclude the possibility of a chemical change in the protein.

Summary

A proteinase was extracted from patent flour, but not successfully purified thereafter. Examination of its behavior toward oxidizing and reducing agents has led to the conclusion that it is an enzyme of

the papain type. There is no reason at present to believe that it is different from the enzyme obtained previously from bran and whole wheat. The flour proteinase was found to be activated by cysteine and inactivated by iodoacetic acid and a variety of bread improvers.

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BOOK REVIEWS

Modern Cereal Chemistry. By D. W. Kent-Jones. Third edition. The Northern Publishing Company, Ltd., Liverpool, England. 724 pages. Price in United States $7.15, at Broomhall's Agency, 230 Produce Exchange, New York.

To all who are interested in keeping thoroughly informed and up-to-date in the rapidly expanding field of cereal technology, the appearance of Dr. Kent-Jones' third edition of *Modern Cereal Chemistry* is an event of major importance. From a modest beginning in 1924, the first edition of 324 pages has now been expanded into a comprehensive, authoritative, systematic, and up-to-date treatise of 724 pages.

Most of the material has been revised, expanded, and rewritten, and four new chapters have been added. Especially timely is the chapter on "Dough Testing Machines," dealing with modern physical methods used in flour and dough testing. Another noteworthy addition is the chapter on "The Microbiology of Cereals," contributed by Dr. A. J. Amos. Two other important new features are chapters, respectively, on "Flour for Purposes Other than Bread Making" and "Cereal and Balanced Rations for Live-stock."

In a large chapter dealing with "Methods of Analysis" the author has fully and adequately described and discussed the details, purposes, and merits of the principal chemical methods, with their modifications for special requirements, that are now available for use in the cereal laboratory. The baking test, with related matters, is appropriately dealt with in a separate chapter.

The author's thorough familiarity with past as well as with current literature in the field of cereal technology is amply proved by his references and bibliography containing approximately 600 citations, which have been selected with care, and which include papers published in 1938. Some will take issue with the selection of certain papers and with the omission of others, but all will agree that in the main an excellent sense of discrimination has been shown.

The book is written in simple, clear, and understandable English, and in a style which reflects the ability, energy, and enthusiasm of the author. Although some may be inclined to criticize the emphasis on certain processes and to question some interpretations placed on various features of flour and dough behavior, it is the opinion of the reviewer that controversial issues are for the most part handled with sound judgment and with a degree of impartiality that is highly commendable. No cereal technologist, whether a beginner or an "old-timer," whether in research or control work, can afford to be without a copy of *Modern Cereal Chemistry*, which the reviewer considers to be the most complete, up-to-date, and authoritative work on wheat and flour technology now in existence.

M. J. Blish

Das Roggenmehl. By Arne Schulerud, Oslo. Published by Verlag von Moritz Schaefer, Leipzig. 149 pages. Price 9.5 marks.

This book on rye flour will be welcome to anybody who is interested in this cereal. In a concise form it gives the present status of our knowledge of rye flour and its baking characteristics. The subject matter is divided into the following six chapters:

I.	Rye varieties and rye flour milling	7 pages
II.	A review of the chemistry of rye flour	28 "
III.	Development and physical structure of the dough	38 "
IV.	The influence of chemical and physical factors on the characteristics of rye flour	27 "
V.	Bacterial action and yeast fermentation	15 "
VI.	What happens during the rye flour baking process	32 "

Chapter I. Supplies statistical data regarding rye culture over the world and a discussion of the rye types and their characteristics in different countries. There is very little mentioned about the rye milling process.

Chapter II. Deals with the importance of the following chemical factors: moisture, ash, protein, fat, and carbohydrates in rye flour. The carbohydrates are especially emphasized on account of the important role they play in the rye baking process. Staling is shortly discussed and attention is called to the importance of acidity. A discussion of the principal enzymes in rye flour closes this chapter.

Chapter III. Contains a discussion of the physical properties of a rye dough, such as consistency, elasticity, plasticity, viscosity, etc. The application of the farinograph to rye doughs is discussed. There is also an interesting treatment of the viscometric phenomena of the rye flour water system, when subjected to gradually increasing temperatures. The possibilities of the Brabender amylograph are discussed in this connection.

Chapter IV. Deals with the influence of salts and some organic acids on rye doughs, also the effect of heat and enzymatic action on the baking quality of rye flour.

Chapter V. Contains a general outline of the happenings in sour dough and yeast-fermented doughs and a discussion of the connection between gas production, yeast quantity, and available sugar.

Chapter VI. Treats of the more technical phases of the rye bread baking process, such as mixing, kneading, dividing, and molding. This chapter contains an interesting description of the processes occurring in the fermenting dough and what happens when the dough is subjected to the heat of the oven until it is ready to be taken out in the form of bread.

A list of 61 references from the literature closes the book. Its author is an outstanding authority on the subject of rye and rye baking and the prospective reader may be assured that he will be in reliable company. The publisher comes in for praise as regards the general appearance of the book.

J. T. Flohil